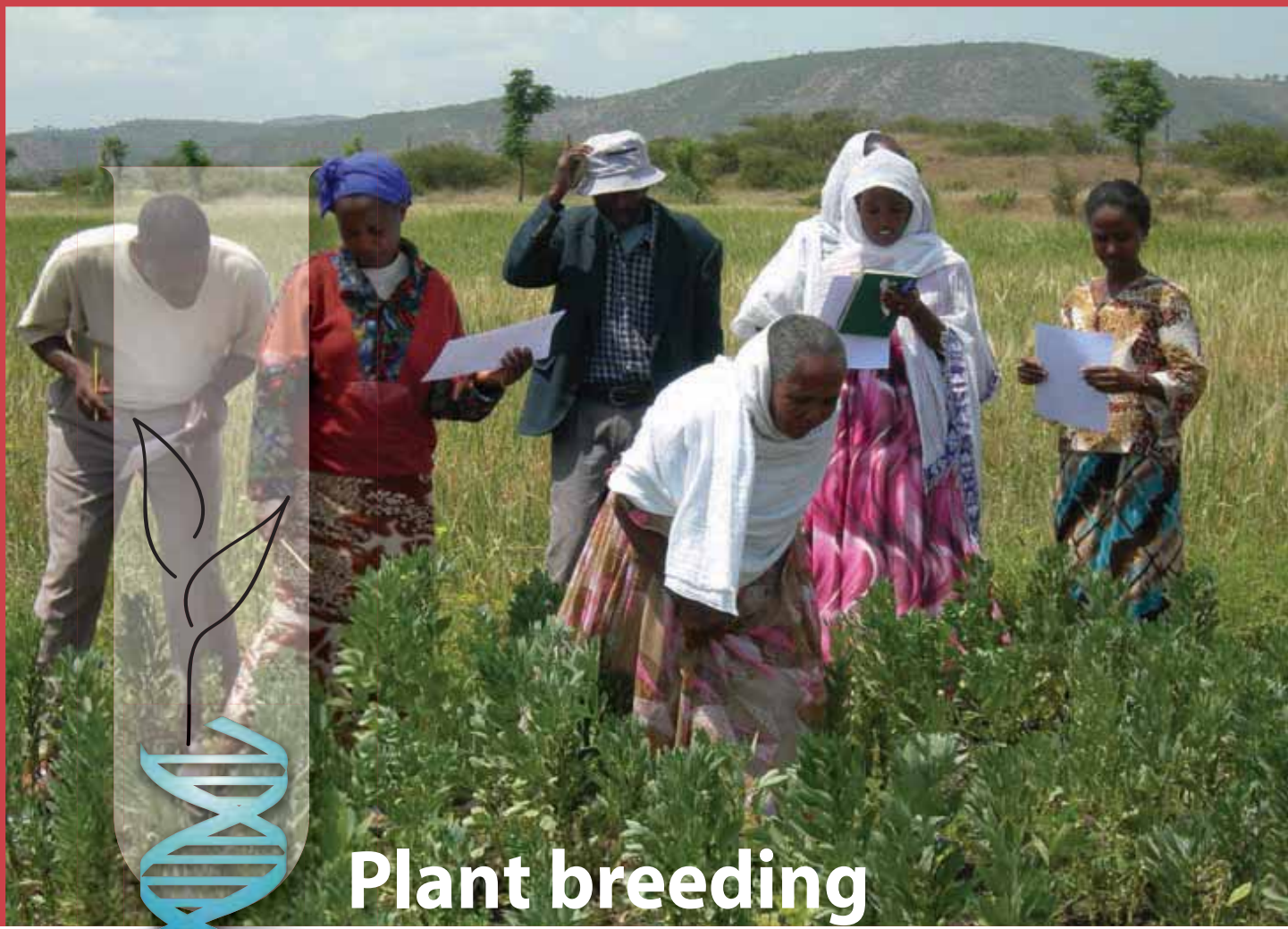

Plant breeding
and farmer participation

ICRISAT

ICARDA

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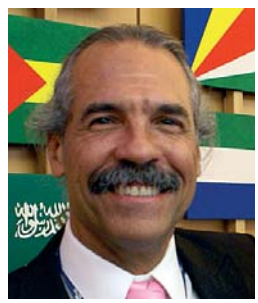
Plant breeding and farmer participation



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Contents

Foreword

Participatory Plant Breeding (PPB) originated in the early 1980s as part of a movement promoting the concept of participatory research, in response to criticisms of the failure of post-green-revolution, experiment-station-based research to address the needs of poor farmers in developing countries. Rooted in debate over the social consequences of the narrow focus of the scientific type of research, PPB gained recognition as an activity mostly promoted by social scientists and agronomists based in anti-establishment non-governmental organizations (NGOs). In consequence, rather than being perceived from the beginning as an additional option available to breeders, PPB for a long time had the image of being one of two contrasting types of plant breeding, with PPB being more "socially correct" than conventional plant breeding.

Even now, nearly thirty years later, this view is still common. Few professional breeders accept that farmers can be full partners in a plant breeding programme, even though everyone agrees that it was farmers that domesticated crops about 10 000 years ago and, in some regions of the world, continued to modify and manipulate them to the present day. Even before the re-discovery of Mendel's laws of inheritance, the work of a number of amateur breeders become an inspiration for Darwin's theories. In several respects, the relationship with farmers on which PPB is based is similar to the ways in which plant breeders worked with producers in North America and Europe in the early twentieth century. At that time it was commonplace for breeders to spend time interacting with producers, and to test new materials collaboratively in farmers' fields in order to understand what producers considered to be desirable traits for an improved variety. However, the combination of industrialization of agriculture and formal training for plant breeders created a gap between breeders and farmers, a gap that was exported to developing countries in the post-war era. As the profession of plant breeding lost the habit of interacting closely with producers, concern for how to address farmers' needs and constraints fell by the wayside. PPB revived this as a central issue, because by the late 1970s it was increasingly evident in developing countries that post-green-revolution "improved" varieties were too often failing to satisfy farmer requirements and were being shunned.

Today there is widespread recognition that the conventional package of new varieties and external inputs, while successful in the more favourable production areas, has often failed to benefit small-scale farmers in marginal areas. As a result, the vital role of PPB as an additional strategy is better understood. Experience has taught that PPB is complementary to conventional plant breeding rather than an alternative type of plant breeding. Demand for a complementary approach has expanded considerably because of pressure to ensure the relevance of research to poor farmers and their diverse agricultural systems, and because PPB allows selection for the specific adaptation required for such a diversity of target environments. Today, about 80 participatory breeding programmes are known worldwide, involving various institutions and various crops. In 2000, an international review of plant

breeding research methodologies concluded that PPB should be an "organic" part of every plant breeding programme aimed at benefiting small-scale farmers in difficult, high-risk environments. In fact, traditional farming and low-input systems, including organic agriculture, are a very heterogeneous population of target environments and not easily served by centralized, conventional plant breeding.

The book demonstrates that PPB is in essence no different from conventional plant breeding, being based on the very same principles of Mendelian, quantitative and population genetics, and therefore has complemented the traditional approach to plant breeding with a number of chapters addressing issues specifically related to the participation of farmers in a plant breeding programme.

The authors of the various chapters have been carefully selected to represent three groups of scientists: the first comprises internationally recognized experts in genetics as related to plant breeding, and in the various aspects of plant breeding (from general methodological issues to more specific issues, such as breeding for resistance to biotic and abiotic stresses, high yield potential, molecular breeding and genotype × environment interactions); the second group is represented by professional breeders who have actually practised participatory plant breeding with a number of different crops and in a number of socially and climatically different areas, using the range of methods presented by the first group; and, finally, the third is represented by a group of scientists with specific expertise in areas not usually covered in classical plant breeding books, such as variety release mechanisms, seed diffusion, institutional issues associated with PPB, and intellectual property rights. A chapter documenting the impact that participatory plant breeding has had after about thirty years of practice has been chosen to be the logical conclusion of the book.

The book is aimed at plant breeders, social scientists, students and practitioners, with the hope that they all will find a common ground to discuss ways in which plant breeding can be beneficial to all and can contribute to alleviate poverty.

Finally, we would like to acknowledge everyone who has, directly or indirectly, contributed to the book: the CGIAR Participatory Research and Gender Analysis Program (PRGA) for the initial idea of producing such a book, the contributors of the chapters for sharing their scientific experience and for enduring a number of revisions of their respective chapters, Dr P.G. Rajendran for his help in the initial editorial efforts and the Directors-General of our Institutions for their continuous support. Final editing and preparation for publication was done by Mr Thorgeir Lawrence.

Abbreviations and acronyms

AB-QTL	Advanced Backcross QTL Analysis
AFLP	Amplified fragment length polymorphism
AMMI	Additive main effects and multiplicative interaction
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
AOSCA	Association of Official Seed Certifying Agencies
ABS	Accelerated Breeding System [for sweet potato]
ASSINSEL	International Association of Plant Breeders for the Protection of Plant Varieties
AVP	Asexually or vegetatively propagated
BC_n	Back-cross generation *n*
BLUE	Best Linear Unbiased Estimate
BLUP	Best Linear Unbiased Prediction
BPE	Before present era
BSA	Bulked Segregant Analysis
Bt	*Bacillus thuringiensis* [gene]
BYDV	Barley Yellow Dwarf Virus
CBD	Convention on Biological Diversity
CBP	Centralized breeding programmes
CCN	Cereal cyst nematode
CE	Common era
CGIAR	Consultative Group for International Agricultural Research
CIDA	Canadian International Development Agency
CIE	Commission Internationale l'Eclairage
CIAL	Local agricultural research committees [in Latin America]
CIAT	International Center for Tropical Agriculture
CIMMYT	International Wheat and Maize Improvement Center
CIP	International Potato Center
CPB	Conventional plant breeding
cv	Cultivar [= cultivated variety]
DArT	Diversity Arrays Technology
DBP	Decentralized breeding programmes
dES	Diethyl sulphate [a mutagen]
DF	Degrees of freedom
DH	Doubled haploid
DHPLC	Denaturating high performance liquid chromatography
DM	Dry matter

DPBP	Decentralized-participatory breeding programmes
DUS	Distinctness, Uniformity, Stability
DW	Dry weight
ELISA	Enzyme-linked immunosorbent assay
EMS	Ethane methyl sulphonate [a mutagen]
EPA	Environmental Protection Agency [United States of America]
F_n	Filial generation *n*
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration [United States of America]
FFS	Farmer field school
FIPAH	La Fundación para La Investigación Participativa con Agricultores de Honduras
FK	Farmer knowledge
FPB	Formal plant breeding
FR	Farmers' Rights
FV	farmer variety [± locally selected]
G×E	Genotype × Environment (Interaction)
GGE	Genotype main effect (G) plus Genotype × Environment (GE) Interaction
GIS	Geographical Information System
GMO	Genetically modified organism
GURT	Genetic Use Restriction Technology
G×L	Genotype × Location
G×Y	Genotype × Year
HPLC	High performance liquid chromatography
IAEA	International Atomic Energy Agency
IARC	International Agricultural Research Center
ICARDA	International Center for Agricultural Research in the Dry Areas
ICP	Inductively coupled plasma [mass spectrometry]
ICPOES	Inductively Coupled Plasma Optical Emission Spectrometer
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
ID	Inbreeding depression
IDRC	International Development Research Centre [Canada]
IFPRI	International Food Policy Research Institute
IITA	International Institute of Tropical Agriculture
INCA	National Institute for Agricultural Science [Cuba]
IP	Intellectual property
IPGRI	International Plant Genetic Resources Institute [now Bioversity International]
IPM	Integrated pest management
IPR	Intellectual Property Rights
IRRI	International Rice Research Institute
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
LD	Linkage disequilibrium

LD_{50}	Lethal dose killing 50% of target
LD_{100}	Lethal dose killing 100% of target
MAS	Marker-assisted selection
MCA	Multiple correspondence analysis
MET	Multi-environment Trials
MFN	Most favoured nation
MNU	Methylnitrosourea [a mutagen]
MRRS	Modified reciprocal recurrent selection
MS	Mean square
MTA	Material Transfer Agreement
MV	Modern variety
MVD	[FAO/IAEA] Mutant Varieties Database
NARS	National agricultural research system
NDVI	Normalized Difference Vegetation Index
NERICA	New Rice for Africa
NIL	Near-isogenic line
NIRS	Near Infrared Spectroscopy
NIR	Near-infrared [spectrum]
OECD	Organisation for Economic Co-operation and Development
OFSP	Orange-fleshed sweet potato
OPC	Open-pollinated cultivar
PBK	Plant breeder knowledge
PBR	Plant breeder's rights
PC	Principal components
PCR	Polymerase chain reaction
PGR	Plant genetic resources
PPB	Participatory plant breeding
PRA	Participatory rural appraisal
PRGA	[CGIAR] Participatory Research and Gender Analysis [Program]
PRI	Photochemical reflectance index
PSD	Participatory seed dissemination
PVP	Plant Variety Protection
PVS	Participatory varietal selection
PVX	Potato virus X
PVY	Potato virus Y
QTL	Quantitative trait locus
R&D	Research and development
RAPD	Random amplified polymorphic DNA
RCB	Randomized complete block [experiment design]
REML	Restricted Maximum Likelihood
RFLP	Restriction fragment length polymorphism
RFSRS	Reciprocal full-sib recurrent selection
RIL	Recombinant inbred line

RRS	Reciprocal recurrent selection
S_n	Selfed generation *n*
SD	Standard deviation
SE	Selection environment
SE	Standard error
SFNB	Spot form of Net blotch
SMTA	Standard Material Transfer Agreement
SNP	Single nucleotide polymorphism
SPCSV	Sweet potato chlorotic stunt virus
SPFMV	Sweet potato feathery mottle virus
SPVD	Sweet potato virus disease
SR	Simple Ratio Vegetation Index
SS	Sum of squares
SSR	Simple sequence repeat
SSTW	Small-scale Third World
TGV	Transgenic crop variety
TILLING	Targeting Induced Local Lesions In Genomes
TLC	Thin-layer chromatography
TPE	Target population of environment
TPS	True potato seed
TRIPs	[Agreement on] Trade-Related Aspects of Intellectual Property Rights
UPOV	International Union for the Protection of New Varieties of Plants
USDA	United States Department of Agriculture
UV	Ultraviolet [radiation]
VCU	Value for Cultivation and Use
VIS	Visible spectrum
WARDA	Africa Rice Centre [formerly the West Africa Rice Development Association]
WI	Water index
WIPO	World Intellectual Property Organization
WTO	World Trade Organization
WUE	Water-use efficiency

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CHAPTER 1

Crop domestication and the first plant breeders

Stan Cox

1.1 INTRODUCTION

If the story of modern humans from the beginning to the present day could be compressed into a feature-length movie, the era of crop domestication would occupy a scene approximately six minutes long, starting about ten minutes from the movie's end. During that scene, the action would be scattered and sporadic; the domestication of any individual crop species would almost always occur in only a single locality and occupy only about 15 seconds to 2 minutes of the film.

In that brief era, in those rare places where today's crops were born, every farmer was a plant breeder. And through succeeding millennia, as agriculture spread across the surface of the planet, much of settled humanity came to participate in plant breeding.

Studies of ancient artefacts and botanical remains, ancient DNA, phytoliths, living plant populations, and the agricultural practices of surviving indigenous societies have converged to provide us with a vivid but still incomplete account of the first plant breeders' genetic revolution. Conventional wisdom based on those studies tells us that domestication was preceded by a period of archaic cultivation, during which people encouraged the growth of particular species and harvested their seed or other plant parts; that when people began to sow a portion of their harvested seed, they selected—automatically and unconsciously—for genes of domestication, such as those curtailing seed dispersal and dormancy; and that, as our ancestors developed a mutual dependency with domesticated plants, they became intentional and versatile plant breeders, selecting for a wide range of desired traits in species grown for grains, roots, tubers, fruits, vegetables or fodder.

Conventional wisdom usually gains its status by being accurate in its generalities but off the mark in some of its specifics. As we will see, that is the case with crop domestication. My purpose in this chapter is not to summarize the 'where' and 'when' of domestication, species by species, nor is it to analyse theories on the origins of agriculture. Those tasks would entail the boiling-down, if not the over-cooking, of a vast and fascinating literature (e.g. see Zeder *et al*., 2006; Sauer, 1993; and Harris and Hillman, 1989). Rather than attempt to summarize that literature, I briefly tabulated in Table 1.1 what is believed to be true, both geographically and chronologically, about the domestication of today's major crops.

Keeping in mind that humanity's brief experiment with domestication involved people in every quadrant of the globe, I will concentrate on the 'how' and 'why' of domestication, on questions about the first plant breeders themselves and the species they transformed: Why did they domesticate some species and not others? How did their farming practices change gene frequencies in plant populations? How long did domestication take? Why did people select for particular traits: unconsciously, intentionally or indirectly? How did their actions affect the genetic structure and diversity of today's crop species? And, finally, what kinds of skills and knowledge did they pass down to the farmer-breeders of more recent times?

Any effort to answer those questions must draw upon examples from the available literature, in which today's major crops, largely cereals and grain legumes, feature most prominently. Although no set of examples can represent the full geographical and botanical range of domestication, I have attempted to rely upon those people,

TABLE 1.1
Species domesticated in each of eight world regions, with approximate age of the oldest evidence of domestication

Region	Species	Common name	Age of the oldest evidence of domestication (years BPE)
West Asia	*Hordeum vulgare*	Barley	10 500
	Triticum turgidum	Emmer Wheat	10 500
	Cicer arietinum	Chickpea	9 500
Africa	*Sorghum bicolor*	Sorghum	8 000[a]
	Pennisetum glaucum	Pearl Millet	?(1)
Eurasia	*Brassica campestris*	Rape	3 500
East Asia	*Oryza sativa*	Rice	7 000
	Glycine max	Soybean	4 000
New Guinea	*Musa* spp.	Banana	7 000(2)
	Saccharum officinarum	Sugar Cane	?
South America	*Ipomoea batatas*	Sweet Potato	4 500
	Arachis hypogaea	Groundnut	4 500
	Solanum tuberosum	Potato	4 500
	Manihot esculenta	Cassava	4 500
	Phaseolus vulgaris(3)	Common Bean	7 500
Mesoamerica	*Zea mays*	Maize	7 500
	Gossypium hirsutum	Cotton	7 500
North America	*Helianthus annuus*	Sunflower	3 000

NOTES: (1) Wendorf *et al.* (1992) found archaeological evidence that wild millet and sorghum were being used in the Sahel 8000 years before present. The sorghum specimens showed evidence that they were in the process of domestication. (2) Denham *et al.,* 2003. (3) Independently domesticated in Mesoamerica as well. Species listed are among the world's 20 most widely grown crops, on a land-area basis (FAO, 2005). Information is from Sauer (1993) unless otherwise indicated.

places and plants that best illustrate the important features of domestication.

1.2 SELECTION AMONG SPECIES

There is little doubt that certain species were 'pre-adapted' (Zohary, 1984) for domestication. Either entire populations or individual plants within populations had to attract the attention of humans before they could be manipulated. With exceptions, plants or populations that exhibited unusually large or numerous edible parts; self-pollination (in sexually propagated species); ease of propagation (in vegetatively propagated species); or delayed seed dispersal (e.g. chickpea: Ladizinsky, 1979) caught the eyes of early cultivators. Bar-Yosef and Kislev (1989) listed characteristics of certain wild cereals (relative to other wild plant species) that attracted early west Asian domesticators: larger grain, local abundance, annuality, lower seed dormancy, diploidy, harvestability and relative ease of seed dehulling.

A common characteristic among crop ancestors was their weediness: their tendency to thrive in disturbed, fertile soils like those associated with human habitation. The circumstances of domestication are, of course, different for every species. In some places, people started out by harvesting conveniently large stands of annual grasses; in others, variations on the so-called 'rubbish heap' theme were at work (Hawkes, 1969). Many crop ancestors were just as responsible for seeking out humans and human-made environments as were people for tracking down the plants. Indeed, according to Hawkes (1969), it "must have seemed little short of miraculous to find that plants needed for food sprang up by their very huts and paths".

In west Asia, however, those destined to become the first agriculturists tended to make their homes near reliable water sources, whereas they gathered wild grains from stands that were often some distance away (Willcox, 2005). Also relying on the west Asian domestication experience, Abbo *et al*. (2005) labelled the rubbish-heap hypothesis 'environmental determinism' that "tends to underestimate the role of human initiative in the Neolithic transition".

One thing is certain: the original domesticators did not adopt just any species that showed up at their doorstep. Then, as now, people had strong ideas about the usefulness of some plant species and the unacceptability of others. Plants with the most to offer were domesticated long ago, while others that were sufficiently weedy, but less desirable, repeatedly presented themselves to humans, only to be ignored or targeted for eradication (Hawkes, 1969).

Prehistoric people gathered and ate foods from a huge range of plant species, but once they began domesticating, it was *annual* plants that they transformed. Among the staple crops in Table 1.1 that yield edible reproductive biomass, the banana is the lone herbaceous perennial. Herbaceous, *grain*-producing, perennial species are not to be found at all among the world's crops plants (Cox *et al*., 2002). Herbaceous perennials generally produce less seed in a season than do annuals. Also, rapid climatic change across the Asian continent at the end of the Pleistocene dramatically increased the availability of those annual, seed-producing species that attracted the attention of cultivators (Whyte, 1977). The difference in seed production between annuals and perennials is a result of contrasting selection pressures during the two groups' evolutionary histories. Selection pressure applied in yet a different direction by plant breeders can increase seed yield and produce perennial grain crops (DeHaan, Van Tassel and Cox, 2005), but only if the right combination of breeding objectives is established.

When we think of how many civilizations built on annual cropping have fallen not to the sword but to the plough (Hillel, 1991; Lowdermilk, 1953) and the soil degradation that continues to haunt agriculture today, we can only lament the fact that the domesticators did not focus more on erosion-resistant perennial species. Apparently, ancient gatherers did utilize the seed of perennial species as food. Weiss *et al.* (2004) identified charred seeds from 3 perennial and 12 annual species of small-grained grasses that people were consuming 23 000 years ago at a site in what is now Israel. Bohrer (1972) discussed traditional methods of harvesting seed from assorted perennial grasses in Poland, Mongolia and North America. Harlan (1989a) listed a wide range of perennial grasses that people living south of the Sahara have harvested for food. Perennial lymegrass (*Leymus arenarius*) was probably cultivated by Vikings before barley reached Scandinavia (Griffin and Rowlett, 1981). Yet no domesticated perennial grain species were handed down to us by the first plant breeders.

Perennials did not compete well with annuals in disturbed soil and would not have followed people back to the fertile, churned soil around their dwellings; if some plants did happen to make their way there, they would have been overwhelmed by repeated disturbance and competition from weedy annuals. More importantly for Neolithic domesticators, farming and plant breeding were one and the same activity. As a result, they inevitably carried plant populations rapidly through sexual cycles, thereby fulfilling an essential requirement of gene-frequency change. Perennial plants re-growing

from vegetative structures would have been much more vigorous than either volunteer seedlings or intentionally sown plants; therefore, even if people tried to cultivate perennials, they would have felt little incentive to sow new generations from seed.

As we shall see, the act of sowing harvested seed applied strong selection pressure. Selection for non-shattering was strengthened when people began tilling new land year after year to sow their seed, perhaps as a part of shifting cultivation to avoid build-up of non-domesticated weeds (Hillman and Davies, 1990). Stands of perennial plants on undisturbed land would have been much less vulnerable to weeds, much more poorly adapted to shifting cultivation, and therefore less susceptible to domestication. One harvest method that spurred selection for seed retention in the annual cereals—uprooting of the plant (Bohrer, 1972; Hillman and Davies, 1990)—is very difficult with most perennials.

Woody perennials of the Mediterranean and west Asia—including olive (*Olea europaea*), grape (*Vitis vinifera*), fig (*Ficus carica*) and date (*Phoenix dactylifera*)—were domesticated in the same region as cereals, but by descendants of the first plant breeders, several millennia after agriculture had been well established (Zohary and Spiegel-Roy, 1975). Fruit-producing trees and vines did not have to compete with annual counterparts for humans' attention. They were vegetatively propagated, and, even today, most sexual progeny derived from them are "not only economically worthless, but often regress towards the mean found in spontaneous populations, showing striking resemblance to the wild form" (Zohary, 1984). The lack of far-reaching genetic changes in Mediterranean tree crops is also manifested in their failure to spread very far beyond their original climatic range, in contrast to annual domesticates from that region (Zohary and Spiegel-Roy, 1975).

Of course, early farmers also practised selection in vegetatively propagated herbaceous species. As with woody species, they selected clones with desirable characteristics – often the results of unusual mutations – and distributed them far and wide. Occasional hybridization or somatic mutation fuelled some continuing selection; for example, spontaneous yam (*Dioscorea* spp.) clones selected for cultivation by present-day farmers in Benin either are wild or are hybrids between cultivars and wild yams (Scarcelli *et al.*, 2006; Mignouna and Dansi, 2003). But with only rare sexual recombination, there was little opportunity for the degree of domestication seen in grain crops (Zohary, 2004).

The earliest plant breeders' disproportionate attention to seed-propagated annual plants has been replicated by most modern students of plant domestication. That preference will be evident in the range of examples on which the following sections draw.

1.3 INITIAL SELECTION WITHIN SPECIES

It is widely recognized that crops were not domesticated simply through gathering or cultivation. Even the most intensive harvesting of cereals does not apply sufficient selection pressure to domesticate a crop fully. Intentional sowing, in contrast, applies strong, unconscious selection pressure (Zohary, 2004). Alleles for non-shattering, lack of dormancy, reproductive determinacy and increased fertility of formerly sterile florets are all favoured by the sowing-harvesting-sowing cycle (Harlan, De Wet and Price, 1973).

In the west Asia of 10 000 years ago, wild cereals grew naturally in large fields of near-monoculture, but they were not a food source that could simply be browsed

at one's convenience. The time between full ripening and total loss of seed through shattering was only a week or two, and with hot dry weather, the period was shortened to two or three days (Zohary, 1969). Gatherers would have needed to be as timely in their harvest as today's farmers, but the harvest season was lengthened somewhat by differences in time of maturity among different cereal species and by elevation differences in the hilly Levant. Staggered harvests would have allowed people to amass large quantities of grain with a relatively long shelf-life. At the heart of the wild cereals' native range, people could obtain reliable harvests from naturally re-seeded stands; it is therefore most likely that the west Asian grain crops were first domesticated at the fringes of their progenitors' distributions (Harlan and Zohary, 1966). It was there that people would have found intentional sowing most helpful in maintaining stands of their proto-crops. At the same time, Willcox (2005) emphasized the patchiness of wild wheat stands throughout the area where emmer wheat was domesticated. People may have felt some incentive to sow seed, thereby initiating domestication, in any productive localities in that area where wild wheat was not already growing.

A study by Hillman and Davies (1990) deserves to be discussed at some length, because it takes into account many of the factors that affect methods and rates of domestication in grain crops. They started by calculating that the rare, recessive mutations for non-shattering that were necessary for domestication of the west Asian cereals were likely to have appeared once every 5 to 20 years in a typical-sized plot tended by an early cultivator. In predominantly self-pollinating wheat and barley, plants homozygous for recessive non-shattering alleles would have appeared the following season. At that point, they write, "farmers gathering their first seed stocks from wild stands will have been totally unaware of the existence of these tough-rachised mutant forms, and they would have remained oblivious of them as long as the crop stayed in its essentially wild state."

Beating spikes or panicles into a basket is the most time-efficient way to harvest wild grain crops (Hillman and Davies, 1990), but it does not apply selection pressure for non-shattering. Harlan (1967) famously collected wild cereals at the rate of 1 kg/hr by hand-stripping of spikes, but that method would not select effectively against shattering either (Hillman and Davies, 1990). Sickling or uprooting ripe or partially ripe crops does apply selection pressure, because it shakes loose some seed from wild-type plants, seed that is lost to the harvester. Hillman and Davies (1990) found experimentally that a consistently low 40 percent of wild-type seed was recovered by sickling or uprooting. Under those conditions, selection would strongly favour genes for non-shattering.

In their simulations, such strong selection intensity, combined with the high degree of self-pollination typical of wheat and barley, would have resulted in complete fixation of a recessive non-shattering gene within 20 to 30 harvest seasons, if people sowed seed each year on 'virgin land'. They further predicted that even if early farmers inadvertently relaxed the selection pressure by harvesting less fully ripened plants or repeatedly sowing on the same land, domestication would have been completed within two to four centuries. It is no wonder that we know so little about the mechanics of domestication, according to Hillman and Davies (1990). If it came and went as quickly as they envisioned it, the process was "unlikely to be preserved on

most Mesolithic or Neolithic [archaeological] sites as a recognizable progression".

Having assumed in their analysis that initial domestication was entirely unconscious, Hillman and Davies (1990) then demonstrated that even if Neolithic farmers had practised intentional selection, they could not have greatly speeded up the process. With conscious selection, people could have done no better than halve the length of time required for domestication, because they could have started selecting only when the mutants were frequent enough to be obvious, perhaps at a frequency of 1 to 5 percent of the stand. By that point, the frequency of mutants had already passed through a lag phase and was poised for a rapid increase in frequency, even under unconscious selection.

What if, because of a thunderstorm or perhaps an excessive delay in harvest, the only intact spikes from which new seed stocks could be recovered were those of mutants? Could domestication have occurred in a single season? Hillman and Davies (1990) discounted this possibility, based on variation in ripening time and the likelihood that birds or other animals would find the isolated spikes before humans did. Nevertheless, any environmental factor that hastened shattering could have increased the selection pressure and speeded up domestication.

Hillman and Davies's argument begs the question of why early cultivators resorted to sickling or uprooting, if beating is the most time-efficient harvest method for wild cereals. They suggested three reasons that sickling or uprooting apparently was preferred at some point: (1) it recovered more seed per unit land area (which, as people became more settled, may have become a more important criterion than seed quantity per unit time); (2) it permitted utilization of the straw for fire-lighting and brick-making; and (3) it may simply have become customary during a series of wet summers when wild cereals did not shatter as readily and the beating method of harvest was inadequate.

When wild cereals of west Asia shatter, their morphologically distinct basal spikelet remains attached to the culm. That spikelet would have been recovered by harvesters who sickled or uprooted plants, but not by those who gathered already-shattered spikelets from the ground. Basal spikelets might also have been left behind by hand-stripping, but that technique requires that grain be harvested before it is fully ripe, to avoid loss through shattering. Among wild barley and wild emmer remains from four archaeological sites greater than 11 000 years old, Kislev, Weiss and Hartmann (2004) found no basal spikelets and a miniscule number of unripe grains. These observations, they maintained, point to ground collecting as the original harvest method among pre-agricultural people of the region. The authors experimented with ground collection, finding that at any time during the region's rainless summer they could pick up large clumps of spikelets by grasping the upward-pointing awns.

Kislev, Weiss and Hatmann (2004) reasoned that after the first autumn rains, ground gatherers would have noticed seedlings sprouting from spikelets, and that sight would have inspired them to sow a portion of their harvested seed. Of course, sowing of ground-collected seed would have selected not *against* but *for* shattering. Kislev, Weiss and Hatmann (2004) do not speculate on how the transition to sowing of non-shattered seed occurred, but a scenario based on their results comes to mind. In collecting seed from the ground, people would have been moving slowly through stands of wild cereals long after full ripening. Any tough-rachised mutant

with its spike still intact atop the culm may have attracted their interest, and they may well have collected it for sowing in a special plot; if that happened, it would have been a very early case of intentional breeding.

Using lentil (*Lens culinaris*) as a model, Ladizinsky (1987, 1993) showed how domestication of west Asian legumes might have followed a sequence different from that of cereals. He noted that wild lentil (*L. orientalis*) plants are tiny, requiring that an estimated 10 000 plants be gathered in order to obtain one kilogram of clean grain. Therefore, lentils could not have been a major part of the gatherers' diet, as were cereals, which could be gathered much more quickly (Harlan, 1967). Furthermore, Ladizinsky argued, there would have been no incentive for sowing; an incipient lentil farmer would have had to sow their entire harvest simply to produce another crop of equal size. That is because each wild lentil plant produces only about ten seeds, of which only one seed on average will germinate the first year, given the seeds' strong dormancy.

Lentils and perhaps other pulses differed from cereals, argued Ladizinsky (1987, 1993), in that at least partial domestication had to *precede* sowing. Through intensive harvesting, people would have drastically curtailed natural reseeding, thereby leaving fields more open to fast-germinating mutants and selecting against seed dormancy. Once dormancy was largely eliminated and people were able to sow seed to good effect, selection pressure for indehiscent, non-shattering pods would have been feasible. But traditional harvesters in southwest Asia uproot lentil plants before full maturity, then sun-dry and thresh them—a process that largely avoids shattering. If that was the harvest method in Neolithic times, selection for non-shattering would have been much weaker in legumes than in cereals.

Zohary (1989) forcefully rejected Ladizinsky's model, arguing that legume and cereal domestication followed very similar paths, starting with cultivation and sowing of the wild progenitors. He maintained that wild lentils can produce not ten, but rather 40 to 70 seeds per plant when well tended in fertile soil; therefore, people might well have found sowing to be worthwhile. Ladizinsky (1989a) responded that the fields of early, inexperienced cultivators would not have been very conducive to high yields, and that conditions would have been more like those encountered by wild legume stands than those in Zohary's (1989) tilled, weeded and well fertilized experiments.

Some researchers have concluded that domestication was a rapid process in the crops they have studied, certainly when compared with evolution through natural selection. Harter *et al.* (2004) estimated that in sunflower, "genetic composition of the domesticates has changed at least 50-fold faster than the wild populations since they diverged." Wang *et al.* (1999) calculated that it took approximately 300 to 1 000 years to completely fix the crucial domestication gene *tb1* that telescopes the lateral branches in maize. Other studies indicate a somewhat slower process. Jaenicke-Despres *et al.* (2003) found that as far back as 4 400 years ago, modern mutant alleles of the genes *tb1*, *pbf* (prolamin box binding factor) and *su1* (starch debranching, which affects tortilla quality) were common. But that was almost 2 000 years after the date of the oldest known archaeological evidence of maize domestication. Based on archaeological evidence from northern Syrian Arab Republic and southeastern Turkey, Tanno and Willcox (2006) argued that "wild cereals could have been cultivated for over 10 000 years before the emergence of domestic varieties", partly because Neolithic cultiva-

tors may have taken care to harvest grain before any of it began shattering. That would have reduced the selection pressure on alleles for non-shattering. Fuller (2007) argued that during the domestication of rice, einkorn and barley, selection for grain size proceeded faster than selection for non-shattering, but that grain-size increases were much slower in pearl millet and leguminous crops. Surveying the archaeological data, he found significant grain-size increases in Asian cereals within a matter of centuries, a result, he reasoned, of the advantage large seeds had when early cultivators sowed them deeply in tilled soil. In contrast, he concluded, shattering was not fully eliminated for 1 000 to 2 000 years.

Gepts (2002) concluded that models based on a few genes can estimate only the minimum duration of the domestication process, whereas archaeological data provide a 'reality check'. Physical remains often indicate that domestication took much longer than would be predicted by genetic models.

Whether farmers' transformation of various wild plants into crops went quickly or slowly, it was not always permanent. False starts on the road to domestication may have been common. At sites in west Asia and North America, groups of people practised relatively intense cultivation of wild progenitors, and even partially domesticated some species before eventually abandoning them; those orphaned plant populations did not contribute to the founding gene pools of today's crops (Weiss, Kislev and Hartmann, 2006). In one dramatic example of that phenomenon, domestic rye may have arisen 10 000 years ago in the Syrian Arab Republic and Anatolia, only to disappear for several millennia before being re-domesticated in Anatolia and Europe (Willcox, 2005).

1.4 THE DOMESTICATION BOTTLENECK AND GENE FLOW

The number of domestication events experienced by individual species has long been a favourite topic of debate among researchers. Blumler (1992) and Zohary (1999) have argued that multiple domestications within a species have happened only rarely. They pointed out that genetic variation is much greater in most wild progenitors than in derived domesticates. They also noted the rarity of parallel domestication in related taxa above the species level. For example, people selected einkorn wheat (*Triticum monococcum*), pea (*Pisum sativum*: Ladizinsky, 1989b), emmer wheat, maize and chickpea from their wild ancestors while leaving sympatric, phenotypically similar, closely related species undomesticated.

Matsuoka *et al.* (2002) detected a single domestication event in maize by analysing microsatellite variation. Based on amplified fragment length polymorphism (AFLP) variation, Heun *et al.* (1997) concluded that einkorn was domesticated only once, in southeastern Turkey, but that result has been challenged on archaeological and climatic grounds (Hole, 1998; Jones, Allaby and Brown, 1998). Willcox (2005) summarized archaeological evidence indicating that einkorn, emmer and barley all experienced multiple domestications.

Noting that evidence for single versus multiple domestication events in Andean crops such as amaranth and peppers is inconclusive, Blumler (1992) cited several factors that render it "seldom if ever possible to rule out multiple independent invention": the progenitor species may have diversified after domestication of the crop; loci used in comparing the wild and cultivated types may be linked to loci affecting traits of domestication or ecological adaptation; or sampling by researchers may be unknowingly

biased. In a simulation study, Allaby and Brown (2003) showed that analyses relying on anonymous genetic markers might provide seemingly conclusive evidence that a species was domesticated through a single event when it was in fact domesticated more than once.

The people of South America and those of Mesoamerica probably took the common bean through two separate domestications (Sauer, 1993). Xu *et al.* (2002) concluded, on the basis of chloroplast DNA variation, that the soybean had a polyphyletic origin, but cluster analysis of nuclear random amplified polymorphic DNA (RAPD) markers indicated that local differentiation of soybean occurred in farmers' fields after domestication was complete (Xu and Gai, 2003). In any case, the soybean passed through a very tight domestication bottleneck (Hyten *et al.,* 2006). Barley is unusual among the west Asian cereals in harbouring a high level of genetic polymorphism. Ladizinsky (1998) concluded that early cultivators must have selected at least 100 non-shattering mutant plants in order to capture the level of variability seen in barley. Because the crop is highly self-pollinated, post-domestication gene flow from its wild progenitor *Hordeum spontaneum* cannot have accounted for the high degree of variability that is evident today (Ladizinsky and Genizi, 2001).

Whatever the initial number of domestication events, it is clear that because of genetic drift the diversity of most crop species is low compared with that of their wild ancestors. Drift results from a genetic 'bottleneck', usually at the point of initial domestication—the well known 'founder effect' (Ladizinsky, 1985). A bottleneck could also be caused by some later event, but generally would have to occur very early in the history of the crop, before people had a chance to distribute it over a large geographical area.

The founder effect often occurred when domestication depended upon rare mutants, but it was most severe when natural amphiploids (doubled interspecific hybrids) were domesticated. A rare amphiploid taken under human care, as was bread wheat, would have represented a gene pool consisting of a single plant—the tightest possible genetic bottleneck (Cox, 1998).

Tenaillon *et al*. (2004) found that loss of diversity in maize relative to teosinte was only 20 percent for putatively neutral loci, compared with 65 percent for loci affected by selection for traits of domestication. They estimated that the bottleneck that caused this mild contraction of variability had a ratio of population size to duration ranging from approximately 2 to 5. That is, the bottleneck population might have consisted of 10 000 plants over 2 000 generations, or perhaps 2 000 plants over 1 000 generations. Based on data from the *Adh-1* locus, Eyre-Walker *et al.* (1998) estimated a bottleneck size/duration ratio for maize of approximately 2; assuming that domestication took 300 years—similar to the duration estimated for einkorn wheat—they envisioned a bottleneck population of only 600 plants.

Sunflower apparently went through a 'substantial' domestication bottleneck, with inbreeding levels of Native American landraces varying from 0.3 to 0.5 (Harter *et al*., 2004). Abbo, Berger and Turner (2003) counted three successive bottlenecks that tightly restricted the genetic variability of the chickpea crop from its earliest days onward: the highly restricted distribution of its wild ancestor *Cicer reticulatum*; the founder effect resulting from domestication; and an early shift by west Asian farmers from autumn to spring sowing of chick-

pea (to avoid crop loss due to the *Ascochyta* blight disease). That shift required selection of plants without a vernalization requirement. This third bottleneck, which, they argue, occurred early in the crop's history, affected chickpea uniquely among the major west Asian crops. However, it reminds us that many species may have passed through bottlenecks caused by intense, early farmer-directed selection for traits other than seed non-dispersal and lack of dormancy.

Haudry *et al.* (2007) found that domesticated emmer wheat showed a 70 percent loss of nucleotide diversity relative to its progenitor *Triticum dicoccoides*. Durum wheat, derived by further selection from emmer, showed an additional diversity loss, for a total loss of 84 percent. Bread wheat's diversity unexpectedly showed only a 69 percent loss relative to *T. dicoccoides*, suggesting extensive introgression from tetraploid wheats during the 8 000 years since the origin of bread wheat.

Finally, we should take note of a much more recent, possibly catastrophic, bottleneck. Clement (1999) documented 138 Amazonian plant species—the bulk of them either fruits, nuts or vegetables—that were in 'an advanced state of domestication' at the time of the first contact with Europeans five centuries ago. Because these species had become to some extent dependent on humans for their propagation, Clement maintains that the cataclysmic post-1492 loss of 90 to 95 percent of the area's human population resulted in an approximate 90 percent loss of genetic diversity in plant species then under cultivation.

Introgressive hybridization between domesticates and their wild or weedy relatives has often expanded genetic diversity, counteracting the effects of the domestication bottleneck. Hybridization among domestic, weedy and wild populations is often an important source of new variation in crops (Harlan, De Wet and Price, 1973; Small, 1984). People tend to remove from a field those weedy hybrids that do not suit their needs, and those weeds tend to be less competitive in the natural environment as well. However, when weeds managed to backcross to crop plants, their less weedy-looking progeny might well have escaped the early cultivator's hand or hoe, remaining in the domesticated population and exchanging genes with it. Weeds often migrate over larger areas than domesticates and jump from one domesticated population to another, exchanging genes along the way (Small, 1984).

Sang and Ge (2007) attempted to reconcile seemingly contradictory evidence regarding the origin of the two rice subspecies *indica* and *japonica* by showing that the current genetic situation could have arisen from either one or two initial domestications, followed by gene flow from the two potential wild progenitors or between the partially domesticated subspecies, or both. It follows, they wrote, that introgression practised by modern plant breeding programmes is, in effect, "the continuation of domestication".

Weeds unrelated to the crop have at times enticed humans to adopt and domesticate them as secondary crops. The ancestors of oats (*Avena sativa*) and rye (*Secale cereale*), for example, caught the eyes of cultivators while growing as weeds in European wheat and barley fields (Holden, 1976).

Through analysis of microsatellites, Matsuoka *et al.* (2002) determined that the genetic diversity of maize was expanded greatly by introgression from teosinte. Wilkes (1977) found maize farmers in the Nobogame Valley of Mexico encouraging the growth of teosinte near and even within their maize fields. They told Wilkes

that the teosinte germplasm makes kernels 'more flinty and stronger'. Nobogame was the only area in which Wilkes found hybridization intentionally fostered, and, interestingly, it was the only place where the flowering times of maize and teosinte were somewhat synchronized. In other areas, people weeded out teosinte, but, at least in Chalco, they fed it to cattle as fodder, then inadvertently returned its seed to the field when applying manure. It is possible that such mechanisms also played a part in the introgression of teosinte genes into maize in the early phases of domestication.

Gene flow into crops has been important in crop evolution, but there is a much larger flow in the opposite direction: from the domesticate into the wild form. That would probably have been the case in Neolithic grain fields as well. Migration of large amounts of wild pollen into fields of self-pollinated crops was limited, and because pollen from the wild conveyed dominant genes for shattering, hybrid progeny were not likely to be collected or planted by farmers (Ladizinsky, 1985). At the same time, there is much evidence that genes regularly migrated out of fields and into wild populations (Ladizinsky, 1985; Harlan, De Wet and Price, 1973). Many studies have estimated hybridization rates by looking for crop-specific alleles in populations of the crops' wild relatives growing at various distances from cultivated fields. They generally find surprisingly high rates, even hundreds of metres away (Ellstrand, 2003).

Differences among crop species in the sizes of their founding populations and subsequent opportunities for gene inflow from the wild have profoundly affected the levels of genetic diversity available to present-day plant breeders. Here, the contrast between bread wheat and grain sorghum is instructive (Cox and Wood, 1999). Hexaploid bread wheat may well have originated from only one or two hybrid plants with genomic constitution ABD (Cox, 1998; Haudry *et al*., 2007). The tetraploid ancestor (AB) had experienced only limited introgression from diploid plants, mostly of the A-genome species. Subsequent gene flow from AB into ABD wheat plants occurred to some extent (Haudry *et al*., 2007), but gene flow from the extremely diverse D-genome donor *Aegilops tauschii* into bread wheat was either non-existent or extremely rare until it was done by twentieth-century plant breeders (Cox, 1998). Therefore, throughout the entire bread wheat species, there is limited genetic variability in the A and B genomes, while its D genome contains only a tiny fraction of the diversity found in *Aegilops tauschii* (Reif *et al*., 2005).

In contrast, people of Africa have always grown grain and fodder sorghum in areas where the crop comes into close contact and interbreeds with wild sorghum races (Doggett and Majisu, 1968). They probably domesticated sorghum in various, widespread locales on multiple occasions, after which it was exposed to a continuous inflow of variability from the wild and weedy gene pools. As a result, grain sorghum today harbours vastly more genetic diversity than does bread wheat (Cox and Wood, 1999).

1.5 GENETIC CONSEQUENCES OF SELECTION

Van Raamsdonk (1995) proposed that most domesticated crops were developed through one of four genetic models (Table 1.2). The models differ in the role of ploidy and the degrees and mechanisms of reproductive isolation. Differences in genetic and

cytogenetic mechanisms meant that the key role of the domesticator varied from model to model (Table 1.2). For instance, with some crops, people functioned as matchmakers, bringing species into contact for the first time; in others, they enforced reproductive isolation.

Domestication tends to intensify the degree of inbreeding in seed-propagated species (Zohary, 2004). The inflorescences of tomato, chili and eggplant (*Solanum melogena*), among other species, were unconsciously selected by domesticators to have shorter styles, which promoted self-pollination (Rick, 1988; Pickersgill, 1969). Artificial selection can push largely self-incompatible populations toward self-compatibility (Rick, 1988), as is believed to have happened in types of *Brassica oleracea*, including summer cauliflowers (Thompson, 1976). Here, there is a kind of ratchet effect: disruption of self-incompatibility systems is easily accomplished, whereas selection in favour of self-incompatibility would have been genetically complex and very difficult (Rick, 1988).

Inbreeding is a powerful accelerator of unconscious selection for traits governed by recessive genes. The fixation of genes for non-shattering that might have required only a few centuries in highly self-pollinated wheat and barley would, with 100 percent cross-pollination, have taken more than 8 000 years (Hillman and Davies, 1990)!

Each of two recessive alleles at different loci in domesticated rice that reduce seed shattering resulted from single-nucleotide substitutions (Li, Zhou and Sang, 2006; Konishi *et al*., 2006). Five of six well studied domestication genes in maize, wheat, rice and tomato exhibit differences in regulatory regions between the wild and domestic alleles

TABLE 1.2

Four models proposed by van Raamsdonk (1995) by which the genetic mechanisms of crop domestication can be classified, along with his lists of crops that exemplify each model and some crucial points at which humans intervened in the domestication process under each model

Domestication model	Examples	Crucial actions by domesticators
Reproductive isolation between a diploid domesticate and its diploid wild ancestor is caused by internal barriers, post-zygotic barriers, external reproductive barriers or apomixis.	Soybean, common bean, chickpea, lentil, cowpea (*Vigna unguiculata*), lettuce (*Lactuca sativa*), citrus fruits (*Citrus* spp.)	Selection for self-pollination and against weedy hybrids; fostering of genetic drift
Development of crop-weed-wild complexes in which genetic information is exchanged more or less freely among diploid domesticates and their sexually compatible wild progenitors.	Maize, rice, barley, grape, sorghum, pearl millet, foxtail millet (*Setaria italica*), radish (*Raphanus sativus*), beet (*Beta* spp.), chili (*Capsicum* spp.), quinoa (*Chenopodium quinoa*)	Adoption of weeds that invade cultivated land; toleration or encouragement of weeds that can backcross to less wild cultigens
One or more rounds of hybridization and polyploidization occur among wild species prior to domestication.	Cotton, sweet potato, groundnut, tobacco (*Nicotiana* spp.), cucumber (*Cucumis* spp.), coconut (*Cocos nucifera*), alfalfa (*Medicago sativa*)	Selection at the polyploidy level
Interspecific hybridization involving at least one domesticated species is followed by polyploidization. Resultant amphiploids are reproductively isolated.	Bread wheat, potato, banana, coffee (*Coffea arabica*), yam (*Dioscorea* spp.)	Bringing formerly isolated plant populations into contact; selection and propagation of rare amphiploid plant(s) found in or near cultivated fields.
In some cases, domestication occurs through a combination of mechanisms from more than one of the above models.	Sugar cane, oat, *Brassica* spp., tomato (*Lycopersicon esculentum*)	—

(Doebley, Gaut and Smith, 2006). Whatever the nature of their mutations, alleles initially selected by domesticators often showed the simplest modes of inheritance. Many genes governing traits of domestication are recessive or additive, and would have been expressed more strongly among the progeny of plants that tended to self-pollinate most frequently. An increased tendency to inbreed may also have been an indirect result of selection for higher grain yield; self-pollination ensures seed and fruit development, especially if the new crop was transported out of the range of its natural pollinators.

Inbreeding also leads to greater within-line uniformity, but it is hard to imagine uniformity being a direct selection criterion for early domesticators, as it would have required that they plant out the progeny of individual plants in separate plots. It is almost certain that they practised mass selection, not progeny testing. But genes promoting self-pollination might have been favoured in very small populations maintained in isolation. Such isolation could have resulted from individual preferences, or perhaps community customs, such as a belief in parts of Guatemala that plants should be grown only from seed produced on the same plot of ground (Pickersgill, 1969). 'Colour coding' (Wilkes, 1989) based on endosperm pigmentation may have helped farmers maintain small, genetically isolated maize populations.

Strong selection to reinforce inbreeding did not occur in crops that were propagated vegetatively; in them, self-incompatibility and out-crossing remained common (Zohary, 2004; Rick, 1988). Through clonal propagation, cultivators could produce large, genetically desirable populations. In contrast to seed-propagated species, in which human selection for improved grain harvests also reinforced meiotic stability, selection in vegetatively propagated species allowed, or even encouraged, variations in chromosomal number and structure, disrupting reproductive development to varying extents (Zohary, 2004).

In a simulation study, Le Thierry d'Ennequin *et al*. (1999) predicted that to fix a full complement of alleles for domestication, either linkage among loci or a significant degree of reproductive isolation is essential. By their models, in predominantly self-pollinating species subject to little migration, people easily fixed alleles at unlinked loci through selection; however, in species with a high degree of out-crossing, human selection favoured blocks of linked domestication genes.

Empirical experiments have demonstrated that linkage among domestication loci is common, regardless of breeding system (Paterson, 2002). In crosses between pearl millet and its wild progenitor *Pennisetum mollissimum*, Poncet *et al*. (1998, 2000, 2002) found linkage among genes affecting spike characters—important components of the domestication syndrome—but not among genes affecting vegetative characters or total grain yield. Burke *et al*. (2002) mapped 78 quantitative trait loci (QTLs) affecting 18 traits in a cross between sunflower and its conspecific wild progenitor. The domestication-associated loci were spread across 15 of 17 linkage groups, but were highly clustered within those groups. Both pearl millet and sunflower are highly cross-pollinated. In rice, a selfing species, QTLs affecting domestication traits also tended to be clustered in linkage groups (Cai and Morishima, 2000).

Wright *et al*. (2005) found that 2 to 4 percent of the genes in maize have probably undergone artificial selection. Much of that selection, especially for the genes involved in plant growth and auxin response that are responsible for the dramatic differences in

plant morphology between teosinte and maize, appears to have occurred during initial domestication. Those growth-pattern genes were clustered, whereas genes affecting amino acid composition were not.

In wild progenitors, significant numbers of agronomically beneficial alleles are often embedded in linkage blocks with other, deleterious, genes. Such desirable alleles tended to be left behind during domestication. For example, in a tetraploid wheat population, Peng *et al*. (2003) found 24 percent of positive QTL effects to be coming from the wild *Triticum dicoccoides* parent. By breaking up such linkage blocks, modern-day breeders can utilize genes that were 'hidden' from early domesticators.

Gepts (2002), surveying studies of domestication traits in maize, pearl millet, common bean and rice, found an average of 2.2 to 5.3 loci per trait. Those loci accounted for only about 50 percent of the total variation per trait, and loci affecting all traits were spread among 3 to 5 linkage groups per species, indicating rather diffuse genetic control. Paterson (2002) found similar patterns in the QTL-mapping literature on sorghum, rice, maize and tomato. He concluded that loci with larger statistical effects were probably biologically significant as well, because they occurred in similar genomic regions in different crop species (Paterson, 2002; Paterson *et al*., 1995).

During domestication, people may have unknowingly favoured plants or populations with a higher inherent rate of recombination per unit of physical chromosomal length. A comprehensive survey showed that mean numbers of chiasmata per bivalent were significantly higher in 46 crop species than in 150 wild species (Ross-Ibarra, 2004). This result was in accord with theory, the bulk of which predicts that an increased rate of recombination is favoured during periods of rapid evolutionary change, of which domestication is an extreme example. Ross-Ibarra found no support for the alternative possibility: that species with higher recombination rates are 'pre-adapted' to domestication.

Even under domestication, the recombination rate is under stabilizing rather than unidirectional selection, because the same high rates that help break up repulsion linkages also speed up the decay of co-adapted gene complexes (Dobzhansky, 1970). Indeed, Ross-Ibarra's comparison of crop and wild species provided evidence for selection against excessive recombination. There are, of course, other mechanisms for maintaining favourable multilocus combinations, including paracentric inversions (Dobzhansky, 1970) and self-pollination (Clegg, Allard and Kahler, 1972).

1.6 INTENTIONAL SELECTION

Although crops were domesticated through largely unintentional selection, there is little doubt that the domesticators quickly became aware of their own ability to change the phenotypic composition of their crops from generation to generation. Genetic modification, once initiated, spread in ever-widening ripples through plant genomes. Sowing spurred unconscious selection for traits like non-shattering; changes caused by unconscious selection prompted observant farmers to practise intentional selection; and intentional selection for one trait often affected other traits as well, through linkage and pleiotropy. Studies of a grain-quality trait in rice show that human selection at a single locus can exert very strong selection pressure on a large chromosomal region surrounding it, causing a so-called 'selective sweep' that can affect other traits much more strongly than would natural selection (Olsen *et al*., 2006).

From the dawn of agriculture until the twentieth century, farmers acted as plant breeders, working almost exclusively through mass selection; that is, by ensuring that some individual plants made a proportionately greater genetic contribution to the following generation than did others. Natural out-crossing would have been frequent enough, even in highly self-pollinating species, to generate useful genetic recombinants. Early plant breeders worked without the benefits of progeny testing or replication, both of which can enhance gain from selection, but they had two other important factors working in their favour: time and ecosystems. Even small gene-frequency changes from year to year translated into large improvements when they continued over vast numbers of growing seasons. And plant populations upon which people exerted gradual selection in a particular locality, through the full range of weather conditions and pest, pathogen, weed and intercrop populations that the locality had to offer, were bound to be resilient and reliable food producers.

When people applied direct selection pressure for some traits, whether intentional or unconscious, they put indirect selection pressure on others. For example, attached glumes increase seed dormancy, so selection for non-dormancy may have increased the frequency of free-threshing plants. Deep sowing may have favoured larger-seeded genotypes (Fuller, 2007), which, in turn, would have had lower grain protein concentrations via dilution. Selection for greater allocation of resources to reproductive growth (higher harvest index) could have increased susceptibility to pests (Rick, 1988). Because plant parts growing from the same meristematic regions exhibit allometric growth, selection to increase the size of one organ generally affected others; for example, selection for larger spikes in the cereals produced wider leaves and thicker culms as well.

Smartt (1969) catalogued the many traits for which early domesticators applied selection pressure in species of *Phaseolus*: a reduced number of lateral branches (to avoid excessive tangling in fields where beans were meant to climb maize plants); more robust leaves and stems; larger flowers; increased photoperiod sensitivity; increased pod and seed size; greater permeability of the testa; and reduced pod dehiscence. However, in examining four cultivated species, he found that not all of those traits were affected in every species.

Chang (1976a, b) noted a similarly increased size of vegetative organs and kernels in rice, along with a more extensive root system; higher tillering capacity; synchronization of tillering; more panicle branches; a longer grain-filling period; tolerance to non-flooded conditions; and loss of pigmentation. However, increases in kernel size and harvest index associated with domestication of rice were less than those in most other cereals (Cook and Evans, 1983). In several species of chili (*Capsicum*), people rejected erect-fruited wild plants in favour of mutants with pendant fruits, which were hidden under the foliage canopy and therefore protected from bird damage (Pickersgill, 1969).

Maize is often recognized as a crop that underwent some of the most remarkable morphological changes during domestication, but, as in most crops, the most obvious transformation was in its reproductive structures. In the words of Iltis (2000),

> *Cover the ears, and it sometimes takes a specialist to tell teosinte from maize … But compare a many-rowed, 1000-grained ear of maize to a 2-rowed, 5-to-12-grained ear of teosinte – and be perplexed! How*

could such a massive, useful monster be derived from such a tiny, fragile, inedible, useless mouse?

Perhaps just as surprising is the finding that morphological differences between maize and its wild ancestor are under relatively simple genetic control (Doebley and Stec, 1993).

Maize is not the only species whose reproductive structures evolved into monstrosities under the guiding hand of early breeders. For example, pearl millet's wild ancestor has heads measuring no more than 10 cm in length, but from it, early breeders selected cultivars with heads up to 2 m long (Harlan, 1989b). In bringing about the visually dramatic domestication of the sunflower, Native Americans selected for the fusion of many smaller heads into fewer, larger ones. People worldwide selected for often dramatically larger reproductive structures in vegetable and fruit crops.

Plant breeding theory, as well as observation of crop domesticates, tells us that the first breeders had their biggest impact on traits that (i) were of the most intense interest to the people who used the plants for food; (ii) were under relatively simple genetic control; and (iii) had a relatively high heritability on a single-plant or single-propagule basis. Therefore, humans altered the appearance and food quality of the harvested product more rapidly than they did traits such as yield per unit area. Contrasting intentional selection with the unconscious selection that preceded and paralleled it, Harlan, De Wet and Price (1973) wrote:

Deliberate selection adds new dimensions to the process [of domestication]. Human selection may be more intense and absolute and is often biologically capricious or even whimsical.

They went on to list a bewildering array of food products and processing techniques, all of which were certain to reveal genetic variation in the crops upon which they were practised.

Human selection for nutritional quality of crop domesticates occurred in the context of other crops that were evolving simultaneously. The most commonly cited example is the complementarity of amino acid profiles in cereals and legumes. Selection among and within species was a matter of health, even life and death. Indeed, Wilkes (1989) declared an 'ethnobotanical rule', stating that when "crops are consumed and not sold, a reasonable level of nutritional adequacy has evolved and been maintained". Neither the single-minded selection for high grain yield per unit area nor the pursuit of high-lysine maize would have occurred to a Mesoamerican farmer of 3 000 years ago.

Plant breeding requires differential phenotypic expression. For example, people could not venture very deeply into the domestication and improvement of a species as a food source if its consumption always resulted in serious illness or death. Indeed, the process by which the sweet almond was derived from its cyanogenic ancestor is still shrouded in mystery (Ladizinsky, 1999). People could begin selecting for lower toxicity once they accomplished at least partial breakdown of toxins through cooking. Other strategies were developed farther back in the human family tree. Geophagy—consumption of clays—is practiced by at least eight primate species (Johns, 1989). People commonly eat clay along with wild potatoes (Johns, 1986) and yams (Irvine, 1952) to de-toxify them, and the practice might have provided latitude for early domesticators to distinguish among different degrees of bitterness without falling too ill too often. Once foods were rendered edible via such practices, selection for lower

toxicity might have been furthered simply through dilution, as people selected for greater root or tuber size (Johns, 1989).

In the potato, there is a remarkable coincidence between toxic thresholds and human capacity for detection. The plant's most common glycoalkaloid is toxic in concentrations above 200 ppm (Johns and Keen, 1986), and tubers with a concentration of greater than 140 ppm are considered unpleasantly bitter by North Americans (Sinden and Deahl, 1976). In contrast, the Aymara Indians of the Andes classify potatoes with concentrations above a range of 200 to 380 ppm as bitter (Johns and Keen, 1986). Because several wild and cultivated *Solanum* species are crucial sources of calories in the Andes, the Aymara and other indigenous people may have developed a taste for somewhat riskier genotypes. Selection for improved nutritional quality can also work against improvement of other traits. For example, potato populations selected for lower glycoalkaloid concentrations had lower resistance to potato leafhopper (Sanford *et al*., 1992).

In a seeming paradox, cyanogenesis (the production of poisonous hydrocyanic acid) is more common in crop plants than in the plant kingdom as a whole. Jones (1998) noted that 16 of the world's 24 leading crop species (by total production) are cyanogenic in some plant part(s) at some stage of growth. Cyanogenesis, Jones observed, is an important mechanism of resistance to pests. People looking to become cultivators, given a wide range of plant species from which to choose, would probably have been attracted to plants that had not already been damaged or largely consumed by other species. Having the unique ability to eliminate cyanogenic glycosides by grinding, steeping and cooking, humans took advantage of plants that could not be consumed by rival species. Reducing the mean toxicity to a safer level allowed them to detect and exploit genetic variation within species.

Toxins aside, the simplification of diet that followed the expansion of agriculture appears in itself to have caused a decline in overall human health (Kates, 1994). Gepts (2002) even implies that had regulatory agencies existed in Neolithic times, domesticated plants might well have failed to receive approval!

Selection for food quality involved more than nutritional considerations. Where muscle and fuel power were resources not to be squandered, genotypes that produced food with lower energy requirements for processing and cooking may have been more highly valued. For example, Harlan (1989b) described how modern cultivators in Mali select sorghum heads with softer grains for ease of pounding, but also keep hard-seeded, more insect-resistant types, for longer-term storage.

In some cases, people may have utilized the progenitor of a crop for one purpose only to find, once they became more familiar with the species, that it possessed one or more other traits that warranted its full domestication. For example, many East Asian plants may have been used for medicinal purposes before being domesticated for food production (Chang, 1970). Bohrer (1972) maintained that the wild grasses that eventually gave rise to cereal crops were originally cut or uprooted for use as animal fodder. However, Hillman and Davies (1990) disputed that idea, arguing that at the time and place of west Asian crop domestication there were no domestic cattle and few domestic sheep or goats. The squash (*Cucurbita pepo*) may have been domesticated first for its seed, or for its hard gourds to be used as containers; once

fleshy vegetable genotypes were selected, people may have stopped growing the gourd types to prevent the appearance of bitter squashes through cross-pollination (Heiser, 1989).

Iltis (2000) concluded that teosinte was first grown by Mesoamericans for its green shoots and sugary pith and not for its grain, which remained enclosed in a hard fruitcase. Later, through increased contact with teosinte as a snack or vegetable, an alert cultivator may have noticed an extremely rare, 'grain-liberating' mutant—on possibly a single occasion—thus kicking off the process of maize domestication.

Amplifying Iltis's hypothesis, Smalley and Blake (2003) suggested and then defended a possible sequence of events by which teosinte domestication proceeded: (1) people began casually harvesting and chewing the sweet stalks and shoots of *Zea* plants; (2) they found that they could extract more juice by mechanical mashing; (3) to preserve the juice, they adopted fermentation techniques already in use with other species; (4) they spread maize far and wide, as a new resource for making alcoholic beverages; and, finally, (5) to expand *Zea* cultivation, they began sowing harvested seed. Once that sequence proceeded as far as step (5)—along with the discovery of the free-kernel mutant—domestication of *Zea mays* as a grain crop would have followed quickly. But the time between its very first utilization by chewing and its full domestication as a grain may have been as long as 2 500 years (Smalley and Blake, 2003).

Perhaps too often, researchers tend to portray the era of crop domestication as one of constant struggle against scarcity and hardship. DeBoer (2003) commented that the possibility of people first having utilized maize for sweet and fermented products.

> *...injects desirous human agents into the account, a palliative for the stern 'food crises' and 'population pressures' that haunt our angst-driven prehistories. How charming it would be to have a snack-and-party crowd, hassled by only an occasional aggrandizer or two, at the base of the Neolithic!*

The initial domestication of crops prompted expansion of farming into new environments, where people continued selection under different conditions, while perhaps repeating the domestication process with new species. Although the ability to accumulate a large excess of grain during a brief harvest season provided, in itself, a strong incentive to settle in one locality for at least a good part of the year [as Flannery (1969) asked regarding a hypothetical community of Neolithic gatherers, "…after all, where could they go with an estimated metric tonne of clean wheat?"], people eventually and inevitably migrated. The ability to take with them a food source that doubled as the means of sowing future crops allowed people to expand agriculture into previously unsettled areas, where the crops encountered new selection pressures and the people encountered new species of plants.

Abandoned fields created by early shifting cultivation in tropical forests may have provided environments in which useful wild plants could survive and grow unusually well, possibly to become domesticates themselves (Piperno, 1989). Barley's early maturity allowed farming at very high altitudes; pearl millet's drought-hardiness extended agriculture into parts of India and Africa that receive 200 mm or less of annual rainfall; and maize brought more people into the sparsely populated, mid-altitude hill country of India and Pakistan (Harlan, 1972). However, once settled in new environments, thanks to a reliable

staple crop, people have not always sought out additional species for domestication; rather, monocultures are common on the fringes of agriculture (Harlan, 1972).

1.7 CONCLUSIONS

In recent decades, institutional plant breeders have come to realize the importance of integrating breeding methodology with farmers' knowledge. Doing so has benefits for breeders—whose selection goals become more embedded in the 'real world'—and for farmers, who come to appreciate better their own ability to change gene frequencies of their crops in favourable directions. This would appear to bring us full circle, to a time like that of agriculture's earliest days, when breeding and farming were fully integrated. But today's agriculturalists also have ten millennia worth of hard-won farming and breeding knowledge on which they can draw by working together.

The first plant breeders lived in prehistoric times, so they left us no direct accounts of the methods they used to domesticate and improve crops. As we have seen, many of our hypotheses about their activities are influenced by our knowledge of the methodologies that farmer-breeders have used in historic times. That is no accident. By extrapolating recent methods back to the origin of agriculture, we are acknowledging a 10 000-year-long, unbroken thread of skills and knowledge that is derived from growing plants for food while simultaneously breeding them for the future. Nevertheless, we should not forget that by coming to rely largely on domesticated plants and animals, we humans have also lost vast amounts of knowledge of other species and ecosystems; there is much that we could re-learn from hunter-gatherer societies of the present, the recent past and the days before agriculture.

Keen observation and use of genetic variation in plant species has been a hallmark of societies that depend directly on those plants, whether the people in those societies were hunter-gatherers, the originators of agriculture, or today's subsistence farmers. As the millennia have passed, knowledge has expanded and methods have evolved, but that thread remains intact. Today's institutional plant breeders also benefit from that accumulated knowledge. Although modern breeders' methodologies are often very different, they are rooted firmly in the past. They also utilize that major part of the first plant breeders' unwritten knowledge that survives in code, the genetic code of the plants themselves.

Had the original crop domesticators been familiar with the principles of genetics, the crop species that they handed down to history might have been even more profoundly transformed. Had they understood the hazards of genetic erosion or pest and pathogen epidemics, they might have domesticated a wider range of species and avoided the genetic bottlenecks that restricted variation in many crops from the very beginning. And could they have foreseen the devastating consequences of soil erosion and water contamination under long-term annual cropping (Cox *et al*., 2006), they might have mounted an effort to domesticate resource-efficient perennial food crops.

Nevertheless, that relative handful of people was responsible for the most important turning point humanity has yet experienced, laying the foundation for the material and cultural world that surrounds us today. But in the evolution of agriculture, it has not been the case that superior knowledge and techniques continuously replace inferior ones. Knowledge survives from every era, all the way back to the origin of crops (and even well before), so that farmers, plant breeders

and all others who work in agriculture can draw upon it in the years ahead.

As it has turned out, the first plant breeders brought about changes in our own species that equal any they achieved with plants, and the plant breeding traditions they established have brought humanity, only in the past century, to a point at which we can study why and how they carried off their revolution, and learn from the answers.

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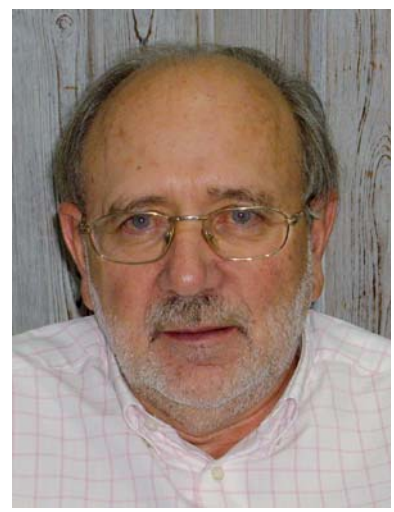
CHAPTER 2

Theory and application of plant breeding for quantitative traits

Javier Betrán, Jesú Moreno-González and Ignacio Romagosa



2.1 A HISTORICAL PERSPECTIVE

A series of accounts from Roman to modern agricultural developments are sketched to show that foundations of current plant breeding, with the significant exception of hybrid breeding, lay in pre-Mendelian times.

2.1.1 Pre-Mendelian plant breeding

Plant breeding traces back to the origin of agriculture (Harlan, 1975; Cox, Chapter 1 this book). Plant domestication through manipulation of few genes with major phenotypic effect generated most food crops early in the evolution of human civilizations. There are approximately 250 000 plant species, of which 50 000 are edible and 5 000 have economic interest, but only 250 are food crops (Sánchez-Monge, 2002). In fact, 90 percent of the calories in the human diet come from just 15 crops, and 60 percent from just wheat, rice and maize. Only a few crops have their origin in the last few centuries and, thus, most major Mediterranean crops are already listed in the Bible. Sugarbeet, celery and rubber became crops in the nineteenth century. Macadamia nut, kiwifruit, blueberries, cranberries, lingonberries and jojoba are examples of the very few post-Mendelian domesticated crops (Ladizinsky, 1998).

Alonso de Herrera published in 1513 his *Agricultura General*, the first modern treatise on agriculture. This text suggests that many current breeding methods for autogamous crops were unknowingly being developed at the turn of our era. Referring to the Roman agronomists, Virgilius, Varro, Plinius and Columella in particular, Alonso de Herrera gave general recommendations about the seeds to be used for sowing cereals. For example, similarly to current bulk selection, he recommended taking the grains from the bottom of the pile upon threshing as they were better because of their heavier weight. As we would currently do to select a genotype within a heterogeneous population using individual or pedigree selection, he suggested that

> *… whenever a plant was found with many large spikes, it should be harvested separately; its seed increased isolated from the rest until a large amount of seed could be used for further growing.*

Johannsen (1903, see Section 2.1.2 below) is considered to be the first to postulate the central plant breeding equation of *Phenotype = Genotype + Environment* (as we will see later, the equation is a little more complex). However, Alonso de Herrera more than 500 years ago stated that "no good crops are to be expected from poor seed unless favoured by good growing conditions". He also recognized the importance of specific adaptation when he wrote that the seed had to be harvested from similar conditions to those where seed was to be grown "from hot to hot, from cold to cold, from dry to dry, from mild to mild, from humid to humid, …".

Plant and animal breeding continued their extraordinary advances in the few centuries before Mendel's work. Spontaneous mutation, hybridization, introgression and crop diffusion played a key role in increasing genetic diversity of crops (Ladizinsky, 1998). Interspecific crosses were first carried out in the eighteenth century. For example, Duchesnes was the first to identify the parentage of the natural hybrid of a new strawberry now named *Fragaria* × *ananassa,* which through continuous breeding became the current big-fruited crop (Darrow, 1966).

Commercial breeding has existed for centuries. Tulip trade in the Netherlands since the beginning of the seventeenth century involved very large amounts of

money (Doorenbos, 1954). The German city of Quedlinburg became known from the middle of the nineteenth century with the establishment of profitable seed companies breeding vegetables and flowers. The Vilmorin Company, a commercial label still in operation, was established in 1743 in France, when Philippe-Victoire de Vilmorin, a horticulturist and Pierre d'Andrieux, a seed collector and botanist of Louis XV, set up the boutique 'Andrieux and Vilmorin' in Paris (www.vilmorin-clause.com). One of the members of the family, Louis de Vilmorin, a contemporary of Mendel, introduced two key techniques in modern plant breeding: first, progeny testing, as a alternative way to assess the value of a given individual based on the phenotype of its offspring rather than just its own; and, second, indirect selection, whereby sucrose yield in the beet root was measured by means of a refractometer. Continuous breeding boosted sugar content from 5–6 percent to 20 percent in just a few decades.

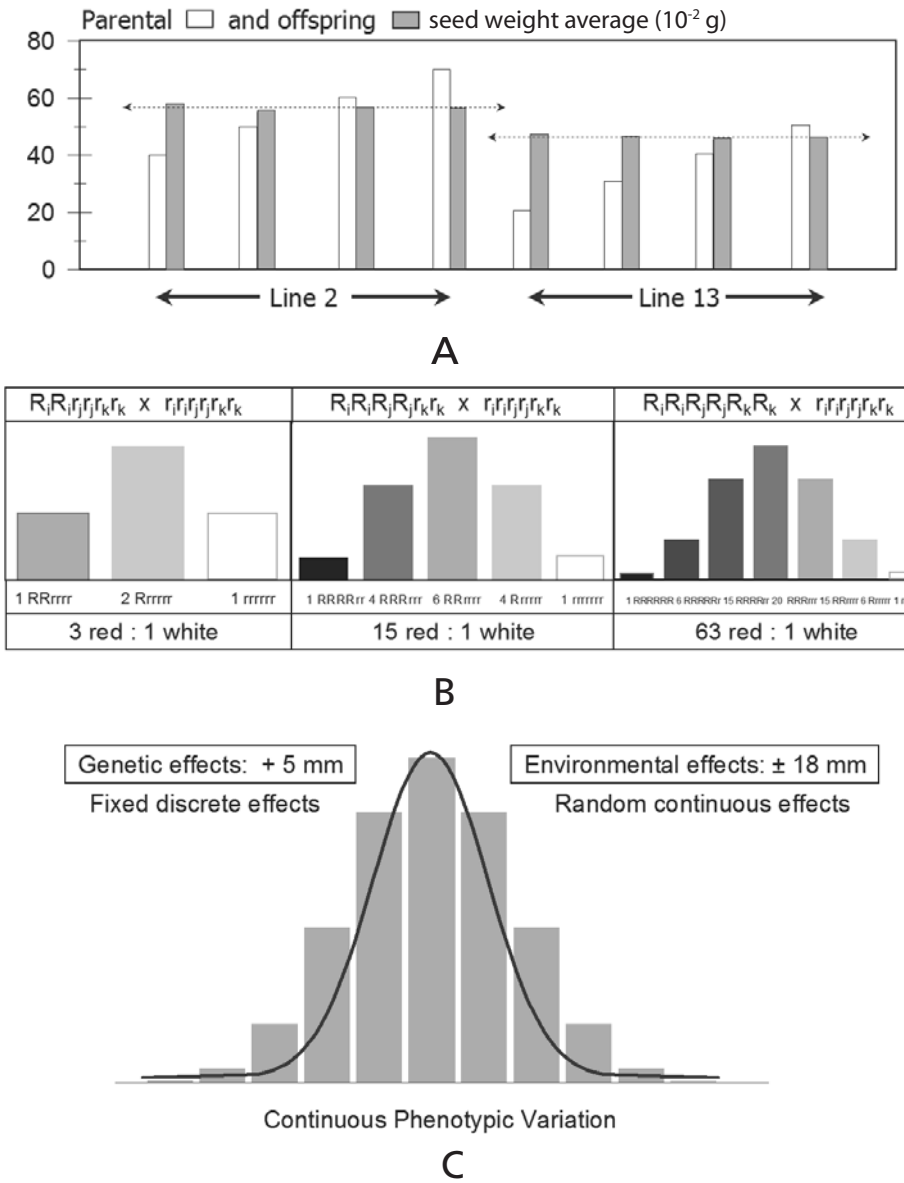
Parallel to plant breeding, by the end of the eighteenth century, animal breeding had developed intensively, based on practical experience. The great success worldwide of the breeders of the Spanish Merino sheep by the leading pioneers in this field such as Robert Bakewell (1725–1795) proved that breeding was an empirical endeavour in which ideas arising out of observation were ahead of academic knowledge. Wood and Orel (2001) summarized the ten intuitive principles and practices used at that time, most of them inadvertently compatible with Mendelian inheritance: (1) The intrinsic nature of an animal (its breed and blood) was the most critical factor determining its form and quality; (2) Good husbandry (mainly diet and housing) were essential for maximizing their intrinsic quality; (3) Transportation of animals from one country to another could be worthwhile, provided that the introduced stock was carefully bred in every subsequent generation; (4) Selective breeding was a powerful agent of change, even for creating new breeds; (5) The more inbred a strain was, the more likely to pass the selected traits to its progeny; (6) Both sexes contributed to heredity, and either could be prepotent, and thus characteristics could be transferred to the opposite sex; (7) Carefully controlled progeny testing was the most efficient way to evaluate an individual's hereditary properties; (8) Selective breeding was applied to single traits or groups of traits; (9) Visible traits could indicate hidden properties; and (10) The value of crossing, as an adjunct to selection, was still a matter of controversy, although the first generation of a cross was becoming accepted for its hybrid vigour.

2.1.2 The onset of Quantitative Genetics: the Mendelians vs. Biometricians debate, and the Neo-Darwinian synthesis

The theory of plant breeding rests on the work of two most influential biologists, Charles Darwin (1809–1882) and Gregor Mendel (1822–1884), and the passionate debate that took place between their followers at the beginning of the twentieth century.

Darwin published in 1859 *The Origin of Species*, in which he persuasively demonstrated that evolution had occurred. He then elaborated the 'Natural Selection' hypothesis to explain the evolutionary process. He backed his observations in nature with the gains of artificial selection achieved in both animal and plant breeding. He strongly supported gradual changes acting over time, rather than discontinuous or abrupt changes. However, he lacked a con-

FIGURE 2.1
Key experimental evidence in the Mendelians vs. Biometricians debate



(A) Johannsen's Pure-Line Theory (1903): heritable (selection between lines) and non-heritable (within a given line) variation for seed weight in beans. (B) Nilsson-Ehle´s Multiple Factor Hypothesis (1909): F_2 segregation ratios for wheat cultivars differing in two alleles at one, two and three loci controlling seed colour. (C) East's experiment on the corolla length in *Nicotiana longiflora* (1916): the apparent continuous variation in the F_2 could be modelled by the superimposition of environmental effects (estimated to be approx. ±18 mm) and genetic effects determined by a reduced number of independent genes (approx. ±5 mm per allelic substitution considering five loci with two alleles at each locus).

vincing theory for the origin of variation and a proper theory of inheritance. Darwin assumed the prevailing model of the blending inherence as observed in progenies of interbred animal crosses by which the attributes of an individual were the result of merging or blending their parent's characteristics (a complete fusion of parental and maternal particles called 'gemmules'). Jenkin (cited by Griffing, 1994) soon realized that under blending inheritance, the variation in the offspring would be halved after each generation of random mating and, thus, variation within any population would be quickly exhausted: if

$$x_o = \frac{x_m + x_f}{2} \quad \text{then}$$

$$\sigma^2(x_o) = \sigma^2\left(\frac{x_m + x_f}{2}\right) = \frac{1}{4}\left(\sigma^2(x_m) + \sigma^2(x_f)\right) = \frac{1}{2}\sigma^2(x_p)$$

where x_o, x_m,, x_f,, and x_p, represent the offspring, male, female and parental values, respectively. New variability should have to be generated to maintain the level of variation within the population.

Mendel's laws of segregation clearly established that any trait is determined by a pair of factors, gametes containing just one of the two factors taken at random. The law of independent assortment establishes that factors from the parents independently combine in the offspring. Mendel supplemented Darwin's Natural Selection because a direct consequence of his laws was that genetic variation could be preserved through time. Whereas genes according to Mendel were conserved over the generations, 'gemmules' received from any parent according to the blending theory were physically lost by being merged together.

Hugo De Vries, contrary to Darwinism, proposed the *mutations theory* for describing the sudden production of new species. This theory was fervently accepted by the Mendelians of the early twentieth century, led by De Vries and William Bateson. They considered the selective value of small variations negligible and believed genetic variation could not be explained statistically. On the contrary, biometricians such as Francis Galton's disciples, guided by Karl Pearson and Raphael Weldon, developed statistical techniques for describing and analysing relationships between relatives with respect to continuous variation. They strongly supported Darwinism and rejected Mendelism as just a series of simple naive general principles. As Griffing (1994) mentions, "a profound controversy developed between the two groups, augmented by the personalities of the scientists involved".

The core of the debate was whether continuous variation observed for metric characters could be reconciled with the discrete Mendelian factors and their inheritance. Mendel's laws and biometrical methods were recognized as complementary after a series of key plant experiments carried out in the first decades of the twentieth century in what Griffing (1994) wisely called the *Era of demystification and reconciliation.* Two important questions were answered: first, What are the basic causes of continuous variation? and, second, What is the nature of the genotypic variation?

Johannsen (1903) addressed the first question with his so-called *Pure Line Theory*. He studied the seed size in a heterogeneous bean variety, 'Princess', a self-pollinated species that was a mixture of pure lines. He proved that continuous selection within a pure-line did not translate into larger grains, but genetic advances could be achieved upon individual selection within a mixture of lines (Figure 2.1A). He

distinguished heritable from non-heritable variability and proposed the concept of *Genotype* and *Phenotype,* which led to the formulation of the central equation in breeding: *Phenotype = Genotype + Environment*.

The Multiple Factor Hypothesis was demonstrated by Nilson-Ehle in Sweden in 1909, answering Griffing's second question. Crossing different wheat varieties of red and white seed colour, he found distinct intensities of red to white kernel 3:1, 15:1 and 63:1 segregation ratios in the F_2 (Figure 2.1B), depending on the red variety used. He postulated that two alleles at each of three independent loci controlled the trait which increasingly showed a continuous phenotypic distribution. East (1916) published the final experiment that clearly brought together Mendelians and Biometricians. He studied the length of the corolla of progenies of two different strains of *Nicotiana longiflora,* which seemed to display blending inheritance. The apparent continuous variation in the F_2 could be explained by the superimposition of environmental effects (estimated to be approx. ±18 mm) on the corolla length determined by a reduced number of independent genes (approx. ±5 mm per allelic substitution considering five loci with two alleles at each locus) (Figure 2.1C).

The crucial work for the development of the Theory of Plant Breeding was published by Fisher in 1918. He introduced the term 'variance' and used its additive properties to partition the phenotypic variance into its components according to a genetic model. He provided the theoretical framework for the final settlement between Biometricians and Mendelians and established the basis of quantitative genetic theory, on which both animal and plant breeding rest. As Griffing says (1994),

> *… the era of the 1920s was blessed by having three of the right scientists (Fisher, Haldane and Wright) with special interest and abilities in the right area (mathematical biology) at the right time.*

They adopted Mendelian inheritance to describe in mathematical terms the basic principles of Darwinian evolution (and, thus, both natural and artificial selection). Their developments constitute the core of the plant breeding theory described below.

As a key component of genetic inheritance, Morgan, Sturtevant, Bridges and Muller clearly demonstrated, working with *Drosophila* during the 1920s, the linear order of the genes in the chromosomes. By 1926, once Morgan's book, *The Theory of the Gene*, was published, it was generally recognized that genetic maps indicating the position and order could be theoretically constructed for any organism (Stebbins, 1994). It has not been until recently, with the advent of molecular markers, that this asseveration has been fully developed. It is also worthwhile mentioning that the concepts of marker-assisted selection (MAS) and quantitative trait loci (QTL) can also be traced to original studies in beans by Sax in 1923 or in tomato by Lindstrom (1926).

Through the use of mathematical models, quantitative genetics studies the genetic architecture and heritability of plant traits, the genetic relationship among them, and the interaction between genotypes and environments. Quantitative genetics thus provides the foundation for the design and utilization of breeding approaches to improve crops. In the next section, we describe the nature of plant traits and genetic phenomena affecting their expression, ways to estimate heritability and subsequent response to selection.

2.2 THE BASIS OF QUANTITATIVE GENETICS

The theory of quantitative genetics has been developed since the early twentieth century to explain the performance, variability and inheritance of quantitative (complex) traits, which are frequently exhibited in living organisms (Fisher, 1918; Wright, 1921; Haldane, 1924; Comstock, Robinson and Harvey, 1949; Kempthorne, 1969; Crow and Kimura, 1970; Falconer, 1981; Dudley, 1982, 1984; Lande and Thompson, 1990). Complex (quantitative) traits behave as non-Mendelian factors and are assumed to be governed by several loci, each with two or several alleles and variable effects.

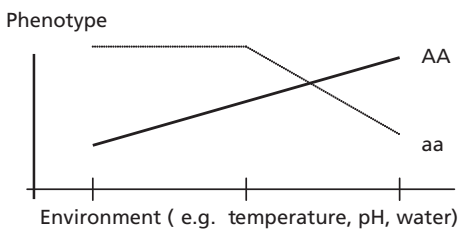
2.2.1 Quantitative traits

Quantitative traits are controlled by many genes, in contrast to qualitative traits that are regulated by one or two genes. It is recognized that a model based on multiple Mendelian factors can explain the continuous variation observed in quantitative traits (Figures 2.1B and 2.1C). For a completely additive system, increasing the number of genes responsible for differences in a given quantitative trait gives a binomial approximation for a normal distribution (Figure 2.1B). Quantitative traits often follow a normal distribution, which is described by a mean and a variance (Figure 2.1C). There are several phenomena that increase the complexity of quantitative traits expression and inheritance, and these are considered below.

The environment influences the expression of quantitative traits

Each genotype may have a norm of reaction (i.e. a range of phenotypes) instead of a single unique expression. Therefore, the same genotypes can have different phenotypes, based on the environmental influence (Figure 2.2).

FIGURE 2.2
Norm of reaction of two genotypes (AA, aa) in different environments



A quantitative trait is usually a composite of many traits, which are governed by many genes with different effects (e.g. grain yield and its components)

Under simultaneous segregation of many genes affecting the trait, the number of possible genotypes increases with the number of loci. If these loci have small effects, distinguishing between the different genotypic classes is difficult. Because the phenotype is the final effect of different loci acting together, many genotypes may have the same phenotype. Under the same assumptions, an increasing number of loci would result in an increasing number of genotypes with the same phenotype.

Furthermore, there are different types of genetic effects among alleles, both within and among loci (i.e. additive, dominance and epistatic effects), which further increase the genetic complexity of quantitative

TABLE 2.1
Number of different genotypes in an F_2 population for a variable number of segregating loci with two alleles

Number of loci	Number of genotypes
1	3
2	9
3	27
5	243
10	59049
...	...
N	3^n

TABLE 2.2
Phenotypic values, genotypes and number of genotypes per phenotypic value (assuming two loci with two alleles each (A/a and B/b) with alleles A and B adding one unit to the final phenotypic value)

Genotype	Phenotypic Value	Number of genotypes per phenotype
AABB	4	1
AaBB, AABb	3	2
AAbb, AaBb, aaBB	2	3
Aabb, aaBb	1	2
Aabb	0	1

traits. Additive effects are the average effect of alleles, which are associated with the number of copies of each allele. Dominance effects originate from the interaction between (among) alleles at the same locus. For a single locus in a diploid species, the comparison between heterozygous loci (e.g. A_1A_2) with the parental homozygous loci (e.g. A_1A_1 and A_2A_2) determines the level of dominance: no dominance (i.e. $A_1A_2 = (A_1A_1 + A_2A_2)/2$), partial dominance (i.e. A_1A_2 is between $(A_1A_1 + A_2A_2)/2$ and A_1A_1 or A_2A_2), complete dominance (i.e. $A_1A_1 = A_1A_2$ when allele A_1 is dominant over A_2), and overdominance (i.e. $A_1A_2 > A_1A_1$ or A_2A_2). Quantitative traits are therefore regulated by many genes having diverse types of genetic effects. More details about genetic effects and their influence in genetic variation and breeding are given by Falconer and Mackay (1996) and Bernardo (2002).

The expression of individual genes is often modified by the expression of other genes (i.e. epistasis)

Epistasis is the interaction between alleles from different genes (i.e. interloci or non-allelic genetic interaction) (Holland, 2001). For two loci, epistasis is the failure of a gene replacement at one locus to remain the same when a gene is replaced at the other locus. Epistasis has strong consequences in plant breeding. First, the consideration of an allele as 'favourable' or 'unfavourable' may depend on the genotype at other loci. Therefore there are favourable and unfavourable combinations of alleles that breeders select for. Epistasis affects the average effects of alleles and dominance deviations and, consequently, the additive and dominance genetic variances (see Section 2.2.2). If epistasis is strong, there can be more heritable variance within lines during selfing and line development than expected. Second, epistasis reduces the correlation between the expression of quantitative traits of early and later selfing-generations. With the presence of epistasis, early generation testing and selection is expected to be less effective than delaying selection until later generations when epistatic effects (e.g. additive × additive epistatic effects) are fixed within lines. Third, epistasis contributes to heterosis and inbreeding depression, although in different manners. While hybrids and population-cross cultivars can exploit all forms of epistasis (additive × additive, additive × dominant and dominant × dominant), only dominance and dominance × dominance epistasis contribute to inbreeding depression (Holland, 2001). Inbreeding depression is heterosis in reverse only when epistasis is absent, but not in the presence of epistasis.

In summary, continuous variation for a quantitative trait is the result of the effect of multiple genetic factors and the environment. Hence, many genotypes can have the same phenotype, and the same genotype can have different phenotypes. The number of loci affecting a quantitative trait, their genomic position, their effects in the final phenotype, the interactions among them, their regulation and the effect of the environment in gene expression is largely unknown for most of quantitative traits (Bernardo, 2002).

2.2.2 Means, variances and correlations for quantitative traits

Genotypic value (G) is the value of a genotype for the trait under consideration. Classically in quantitative genetics, G has been divided into additive, dominance and epistatic effects: G = A + D + I (Falconer and Mackay, 1996). The variation among genotypic values in a breeding population is the genotypic variance (σ_G^2). The breeding value of one individual assesses its usefulness in selection. It is determined by the mean of its progeny and is associated with additive effects. Breeding value in one individual is twice the mean deviation of its outcrossed progeny from the population mean, which is equal to the sum of the average effects of the alleles it carries. The variation among breeding values is attributed to the additive effects of genes and is called additive genetic variance (σ_A^2). Dominance deviation, D, is the difference between the genotypic value (G) and the breeding value (A) for a given genotype in the absence of epistasis. The dominance deviations are due to within-locus interaction between the different alleles. Variation among genotypes for dominance deviations is the dominance genetic variance (σ_D^2). Finally, the variation associated with differences among genotypes for epistatic interactions is the epistatic variance (σ_I^2).

Genetic variance is the sum of the additive, dominance and epistatic genetic variance components: $\sigma_G^2 = \sigma_A^2 + \sigma_D^2 + \sigma_I^2$ (Hallauer and Miranda, 1988). The estimation of additive, dominance and epistatic effects requires knowledge of the allele composition of genotypes and their corresponding genotypic values. The estimation of genetic variance components is conducted using genetic mating designs with a family structure of relatives with known genetic covariance (see Section 2.2.6). Their estimates are used to assess heritability and expected response to selection.

The characterization of genetic properties of quantitative traits (loci effects, intra-locus gene action, epistasis, pleiotropy, linkage, allele frequencies, environmental influence in gene expression, etc.) has been pursued through the study of observable phenotypic properties (mean, variance, resemblance between relatives, correlation among traits, response to selection, inbreeding depression and heterosis). More recently, advances in plant genomics and molecular biology and physiology are contributing to better understand the genetic architecture of quantitative traits and facilitating the connection between genetic and phenotypic properties (Cooper, Podlich and Smith, 2004).

Observable phenotypic properties can be estimated statistically. For two traits (X and Y) measured in several experimental units (i.e. *n* genotypes), means, variances, covariance and correlation can be estimated as follows:

Means:

$$\bar{X} = \frac{1}{n}\sum_{i=1}^{n} x_i \qquad \bar{Y} = \frac{1}{n}\sum_{i=1}^{n} y_i$$

Variance:

$$\hat{\sigma}_x^2 = \frac{1}{n-1}\sum_{i=1}^{n}\left(x_i - \bar{X}\right)^2 = \frac{1}{n-1}\left(\sum_{i=1}^{n} x_i^2 - n\bar{X}^2\right)$$

Covariance:

$$\hat{\sigma}_{XY} = \frac{1}{n-1}\sum_{i=1}^{n}\left(x_i - \bar{X}\right)\left(y_i - \bar{Y}\right) = \frac{1}{n-1}\left(\sum_{i=1}^{n} x_i y_i - n\bar{X}\bar{Y}\right)$$

Phenotypic correlation:

$$r_{XY} = \frac{\hat{\sigma}_{XY}}{\hat{\sigma}_X \hat{\sigma}_Y}$$

Genotypic correlation:

$$r_G = \frac{Cov_G}{\sigma_{G_X}\sigma_{G_Y}} = \frac{\hat{\sigma}_{G_{XY}}}{\hat{\sigma}_{G_X}\hat{\sigma}_{G_Y}}$$

Heterosis is the superior performance of crosses relative to their parents (Falconer and Mackay, 1996). Mid-parent heterosis is the difference between the hybrid and the mean of the two parents (commonly expressed as a percentage):

$$\frac{\bar{X}_{F1} - MP}{MP} * 100 = \text{percentage Mid-parent heterosis}$$

where: $\bar{X}_{F1}$ is the mean of the hybrid; and MP the average of the two parents.
Mid-parent heterosis also may be defined as:

$$\bar{X}_{F1} - MP = \sum_{i=1}^{n} f_i^2 d_i$$

where: d_i is the level of dominance (deviation of the heterozygous from the homozygote mid-parent); and f_i is the difference in allele frequencies among the parents for locus i (Falconer and Mackay, 1996).

Heterosis is dependent on the presence of directional dominance and allele frequency differences. Heterotic groups in cross-pollinated species have been created and enhanced by creating groups and families that differ in allele frequencies in genes affecting target trait(s) (i.e. this increases the value of f_i in the above formula).

Inbreeding comes from mating individuals that are related by ancestry. The consequence of inbreeding is an increase in homozygosity that leads to a depressive negative expression of traits, referred to as inbreeding depression. Inbreeding depression can be expressed as:

$$\bar{X}_0 - \bar{X}_F = 2pqFd$$

where: $\bar{X}_0$ and $\bar{X}_F$ are the mean of the population without and with inbreeding, respectively; p and q are the allele frequencies in the populations; F is the inbreeding coefficient; and d the dominance deviation (Falconer and Mackay, 1996).

The estimation and expression of inbreeding depression (ID) can be calculated as: $\bar{X}_0 - \bar{X}_F$ as the ID in absolute units, where $\bar{X}_0$ is the mean of the trait without inbreeding and $\bar{X}_F$ is the mean of the trait with a given amount of inbreeding F ($0<F<1$).

Inbreeding depression is commonly reported as a percentage:

$$\frac{(\bar{X}_0 - \bar{X}_F)}{\bar{X}_0} * 100 .$$

2.2.3 Genetic linkage and implications in plant breeding

Loci located in different chromosomes are assorted independently. However, loci close together in the same chromosome are not assorted independently: they tend to be inherited together and are considered 'linked loci'. Groups of alleles in loci that are linked and are transmitted together from one generation to the next are called linkage blocks or haplotypes (Figure 2.3). When considering two loci, linkage can be in coupling or repulsion (Falconer and Mackay, 1996). The degree of linkage is measured by the recombination frequency, which determines the proportion of recombinant and parental gametes. If 'R' is the genetic recombination between loci A and B, the gametes frequencies are estimated as shown in Table 2.3.

Genetic linkage has important implications in plant breeding:

TABLE 2.3
Gametic frequencies for two loci with two alleles each (A/a and B/b)

Gamete	Linked		Unlinked
	Coupling	Repulsion	
AB	½ (1-R)	½ R	¼
Ab	½ (1-R)	½ R	¼
Ab	½ R	½ (1-R)	¼
aB	½ R	½ (1-R)	¼

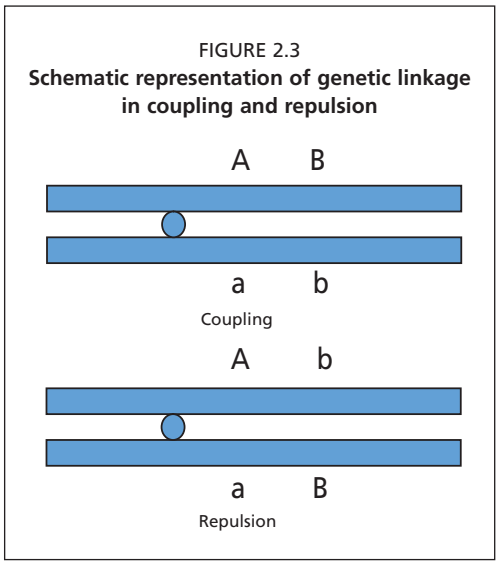


FIGURE 2.3
Schematic representation of genetic linkage in coupling and repulsion

- Selection during breeding is actually applied to linked blocks of genes housing target genes regulating the expression of target traits.
- Linkage is desirable in breeding if favourable or unfavourable alleles are linked in coupling, and undesirable if they are linked in repulsion. The probability of obtaining desirable genotypes in a segregating population is greater than with independent assortment when desirable alleles are in coupling.
- If the genes are in repulsion, large segregating populations will be necessary to break unfavourable linkages by recombination. Heterozygosity is necessary to break up linked genes; otherwise, crossing over does not create new combinations of alleles at linked genes.
- The probability of maintaining desirable linkage blocks in backcross breeding populations is higher than with F_2 breeding populations. At the same time, the probability of breaking up unfavourable linkage blocks is greater in F_2 populations than in backcross populations.

Additive and dominance variance estimates are biased by linkage. Additive variance, σ^2_A, increases with coupling and decreases with repulsion. Dominance variance, σ^2_D, increases in both types of linkage if dominance effects of the linked loci have the same sign. As a consequence, the degree of dominance
(dominance ratio estimated as $\sqrt{4\sigma^2_D / 2\sigma^2_A}$)
increases with repulsion (i.e. pseudo-dominance). This pseudo-dominance decreases with recombination (e.g. applying random mating).

2.2.4 Mating system and population structure

Crop plants are propagated asexually, sexually or both. The mode of reproduction directly affects the population structure of breeding and natural populations of crop species. Asexual reproduction involves either vegetative propagation or apomixis. The cultivars in asexually propagated crops are clones or mixtures of clones. Selection can be conducted among clones to select those most suitable, which are then propagated asexually. Crops that reproduce sexually are self-pollinated, cross-pollinated or a combination. Self-pollination induces reduction of heterozygosity and fixation of alleles in a homozygous condition. Therefore populations and landraces (local or traditional varieties) of self-pollinated crops are commonly a mixture of inbreds, while cultivars are commonly selected pure lines. In contrast to self-pollination, cross-pollination maintains heterozygosity if the populations are big enough and plants mate at random. Therefore populations of cross-pollinated species are a mixture of hybrid genotypes, while cultivars are commonly hybrids or synthetics developed from crossing selected inbreds, or

selected subpopulations from broad-based populations (e.g. pearl millet). Average heterozygosity is much greater in cross-pollinated species than in self-pollinated species. Selfing induces inbreeding depression while cross-pollination induces hybrid vigour. The degrees of inbreeding depression and hybrid vigour are greater in cross-pollinated crops than in self-pollinated crops.

Most of the scenarios in quantitative genetics are studied in populations of genotypes. These populations can be characterized by their genotype and allele frequencies. In large, random-mating populations the gene frequencies and the genotype frequencies have a simple relationship ($p_{ij} = p_i p_j$) and are constant from generation to generation. A population with these characteristics is in Hardy-Weinberg equilibrium (Falconer and Mackay, 1996) and is frequently used as reference framework in Quantitative Genetics modelling. There are several events that cause populations to deviate from an idealized Hardy-Weinberg equilibrium: mutation, genetic drift, migration and selection. Most of these forces are frequently acting together in breeding populations. Small population sizes cause unpredictable alterations in allele frequencies, known as genetic drift. Consequences in breeding are that genetic variation is reduced, and desirable alleles can be lost by chance. Migration can also be used in crops plants through the introduction or introgression of genotypes with increased frequencies of desirable alleles. Selection is the most powerful directional force to change allele frequencies and consequently the expression of quantitative traits. The response to selection depends on allele frequencies of loci regulating the expression of target traits, heritabilities and selection intensities, and the breeding approaches employed.

When considering allele and genotypic frequencies at several loci together there is another type of disequilibrium, known as gametic phase or linkage disequilibrium (LD). LD is defined as the non-random association of alleles at different loci (Bernardo, 2002). For two loci, it is measured as the difference between the observed gamete frequencies and the product of the frequencies of the corresponding alleles:

$$D = p_{(A_iB_j)} - p_{(A_i)}p_{(B_j)}$$

where $p_{(A_iB_j)}$ is the frequency for the A_iB_j gamete, and $p_{(A_i)}, p_{(B_j)}$ the allele frequencies for alleles A_i and B_j, respectively.

High LD exists whenever there is linkage or the population is subject to selection, genetic drift or admixture. It is greatly influenced by several factors, such as population structure, recombination hot spots and the mating system. Genetic recombination between loci in disequilibrium reduces LD. Therefore, LD between unlinked loci in different chromosomes decreases faster than LD between linked loci ($D_t = D_0 (1\text{-}r)^t$, where r = recombination frequency, t = number of generations and D = amount of LD). The tighter the linkage, the longer LD is maintained.

In recent years LD has been used or exploited to associate genomic regions with the expression of quantitative traits, either through artificial LD created by hybridization of contrasting genotypes (QTL mapping) or by using naturally occurring LD in breeding or natural populations (i.e. association genetic studies) (Lee, 1995; Buckler and Thornsberry, 2002; Mackay and Powell, 2007).

2.2.5 The environment and its interaction with genotypes in plant breeding

The environment affects the expression of quantitative traits, and different environments can affect genotypes differently (See Chapter 20). Phenotypic values are classically divided into genotypic (G), environmental (E) and genotype × environmental interaction (G×E) effects: P = G + E + G×E. Likewise, phenotypic variance is divided into genotypic, environmental and G×E variance components: $\sigma_P^2 = \sigma_G^2 + \sigma_E^2 + \sigma_{GE}^2$. In breeding, several genotypes are commonly evaluated in several environments. When genotypes tested differ in their relative performance across environments there is G×E, which can affect response to selection. Non-crossover interaction, i.e. where rank of genotypes does not change across environments, does not have any effect in selection as the best and worst genotypes are the same in all locations. Crossover interaction, where the rank of genotypes changes across environments, has strong consequences in breeding as best and worst genotypes in different environments are not the same (Romagosa and Fox, 1993). There are different breeding strategies that deal with this issue (Annicchiarico, 2002, and see Chapter 20 in this volume). Definition of target and selection environments is critical when allocating resources in a breeding programme. In this process, several decisions have to be made regarding selection for broad versus specific or local adaptation; selection on farm versus research station; and selection under optimal versus stressed conditions (Atlin, Cooper and Bjørnstad, 2001). Cultivars can perform well under a wide range of environments (broad adaptation) or under specific growing conditions (narrow, specific or local adaptation) (Ceccarelli, 1989).

Selection response in the target environment can be expressed as:

$$R_T = \sigma_G i h_s r_G$$

where σ_G = genetic standard deviation, i = selection intensity, h_s = square root of heritability in the selection environment, and r_G = genetic correlation between the selection and target environments (adapted from Bänziger and Cooper, 2001). Thus the effectiveness of a selection environment is determined by the heritability of the traits(s) under selection in that environment, and the genetic correlation between the performance in the selection environment and the target environment (i.e. indirect selection theory, see Section 2.2.8). When the heritability is low or the correlation is low or negative, or both, little progress in the target environment can be expected regardless of who does the selection (farmers or breeders). Multi-environment trials conducted at a large number of sites to adequately sample the target environment maximizes the correlation between target and selection environments, facilitates selection of broadly adapted hybrids or varieties, and exploitation of G×E. Weighted selection strategies, where individual trials found more relevant to the target environments are given more emphasis than less relevant trials, can be used (Podlich, Cooper and Basford, 1999). Increasing the number of environments, as the number of entries decreases during the breeding process, evaluating in environments that disclose genetic variation for the traits under selection, and combining the understanding of the genetic control of target traits and of the target environments, are important components of successful breeding strategies. As we will see later in the book, Participatory Plant Breeding

makes it easier to implement the first and the third of these strategies.

2.2.6 Heritability

Heritability is the relative importance of genetic and non-genetic factors in the expression of phenotypic differences among genotypes in a population (Fehr, 1987). There are two basic types of heritability: broad-sense heritability and narrow-sense heritability (Holland, Nyquist and Cervantes-Martinez, 2003; Nyquist, 1991).

Heritability in the broad sense (H) is the proportion of the phenotypic variance of family means that is due to all genetic effects (Falconer and Mackay, 1996; Holland, Nyquist and Cervantes-Martinez, 2003): $H = h^2_b = \sigma_G^2/ \sigma_P^2$. Broad-sense heritability can be estimated from standard analysis of variances. For example, in the case of genotypes evaluated across several environments, the corresponding analysis of variance and heritability estimate can be illustrated in Table 2.4.

Genotypic and phenotypic variance components can also be estimated using Restricted Maximum Likelihood methods (Holland, Nyquist and Cervantes-Martinez, 2003).

Heritability in the narrow sense (h^2) is the proportion of phenotypic variance among individuals in a population that is due to heritable genetic effects (Nyquist,

TABLE 2.4
Analysis of variance and broad sense heritability estimates in the case of a series of g genotypes evaluated across e environments

Source of variation	Degrees of freedom	Mean Squares	Expected Mean Squares
Environment	e-1		$\sigma_e^2 + g\sigma_{r(E)}^2 + r\sigma_{GE}^2 + rg\sigma_E^2$
Rep(Environment)	(r-1)e		$\sigma_e^2 + g\sigma_{r(E)}^2$
Genotype	g-1	MS_G	$\sigma_e^2 + r\sigma_{GE}^2 + re\sigma_G^2$
Genotype × Environment	(g-1)(e-1)	MS_{GE}	$\sigma_e^2 + r\sigma_{GE}^2$
Error	(g-1)(r-1)e	MS_E	σ_e^2

Notes:

Total phenotypic variance: $Var(Y_{ijk}) = \hat{\sigma}_P^2 = \hat{\sigma}_e^2 + \hat{\sigma}_{GE}^2 + \hat{\sigma}_G^2$

Phenotypic variance of genotypic means: $Var(\overline{Y}_{ijk}) = \hat{\sigma}_{\overline{P}}^2 = \frac{\hat{\sigma}_e^2}{re} + \frac{\hat{\sigma}_{GE}^2}{e} + \hat{\sigma}_G^2 = \frac{MS_G}{re}$

Genotypic variance $= \hat{\sigma}_G^2 = (MS_G - MS_{GE})/re$

Heritability on individual experimental unit basis: $H_i = h_{bi}^2 = \frac{\hat{\sigma}_G^2}{\hat{\sigma}_G^2 + \hat{\sigma}_{GE}^2 + \hat{\sigma}_e^2}$

Heritability on a genotypic-mean basis: $H_m = h_{bm}^2 = \frac{\hat{\sigma}_G^2}{\hat{\sigma}_G^2 + \frac{\hat{\sigma}_{GE}^2}{e} + \frac{\hat{\sigma}_e^2}{re}}$

TABLE 2.5
Relatives, their covariance and regression or correlation values in terms of narrow sense heritability

Relatives	Covariance (w/o epistasis)	Regression (b) or correlation (t)
Parent – Offspring	½ σ^2_A	b = ½ h^2
Midparent - Offspring	½ σ^2_A	b = h^2
Half-sibs	¼ σ^2_A	t = ¼ h^2
Full-sibs	½ σ^2_A+ ¼ σ^2_D	t ≥ ½ h^2

TABLE 2.6
Additive and non-additive variance components in different generations

Generation	σ_A^2	σ_D^2	σ_{AD}^2	σ_E^2
P_1	0	0	0	1
P_2	0	0	0	1
F_1	0	0	0	1
F_2	1	1	0	1
BC_{11}	½	1	- 1	1
BC_{12}	½	1	1	1

Notes: $\sigma_A^2 = (2\sigma\ ^2_{F2} - \sigma\ ^2_{BC11} - \sigma\ ^2_{BC12})$;
$\sigma D2 = (\sigma\ ^2_{BC11} + \sigma\ ^2_{BC12} - \sigma\ ^2_{F2} - \sigma\ ^2_{P1/P2/F1})$;
$\sigma AD = ½(\sigma\ ^2_{BC12} - \sigma\ ^2_{BC11})$

1991; Holland, Nyquist and Cervantes-Martinez, 2003): $h^2_n = \sigma_A^2 / \sigma_P^2$. Narrow-sense heritability can be estimated from variance components or from parent-offspring regression. In both cases, genetic relationships among relatives (lineal (parent-offspring) or collateral (full- or half-sibs)) are used (Table 2.5). The estimation of additive and non-additive variance components is conducted through linear models and proper mating designs (e.g. North Carolina I, II and III, Hallauer and Miranda, 1988) or using information from different generations (Kearsey and Pooni, 1998) (Table 2.6).

Heritability is used to estimate expected response to selection and to choose the best breeding approach to improve the target trait(s). Traits with high heritabilities can be selected on a single-plant basis (e.g. mass selection), faster, and in a low number of environments. In contrast, traits with low heritabilities require selection on a family basis and in a greater number of environments to determine breeding values of genotypes. Heritability estimates for the same trait are variable (i.e. heritability of a trait is not a fixed value) and their magnitude depends on several factors (Fehr, 1987):

- ***Environment***: it is important to have adequate samples of environments from the target population of environments. In addition, estimates for genetic variance should be free of G×E variance.
- ***Reference population***: the amount of genetic variation and inbreeding present in the population affects heritability estimates. Higher inbreeding levels are associated with higher genetic variances and therefore with higher estimates of heritability.
- ***Sample of genotypes evaluated***: genotypes used to estimate heritabilities in one population should be chosen at random. If the sample is not a representative random sample (e.g. selected genotypes), the ratio between genetic and phenotypic variation is called *Repeatability* (Fehr, 1987). Repeatability estimates in a single environment provide a measure of how much of the variation is genetic and therefore is a measure of the degree of precision of data and the ability to detect significant differences among genotypes.
- ***Method of estimation***: heritability of a quantitative trait can be computed by several methods (variance components, parent-offspring regression, etc.) and heritability estimates can differ among them (e.g. heritability on a family basis is greater than on a plant basis; see Table 2.4). Heritability estimates calculated on the basis of selection unit are preferred to estimated expected response to selection.

- ***Generation or progenies***: different progenies exploit different proportions of additive and dominance variances. Inbred progenies have greater heritabilities than full-sib and half-sib families.
- ***Allele frequencies***: heritability is affected by allele frequencies. Therefore any change in allele frequencies (selection, genetic drift, mutation, migration) could change heritability values for the same trait and reference population.

Heritability of a trait can be estimated using the amount of genetic gain that is realized by selection within a population (Falconer and Mackay, 1996). This is known as *realized heritability* and can be estimates *a posteriori* as : h^2=R/S, where R = response to selection and S = effective selection differential applied in selection.

2.2.7 Response to selection

The theoretical response to selection can be defined as $\Delta G = S\ h^2$, where S is the selection differential (the difference between the mean of the selected individuals and the mean of the whole population) and h^2 the heritability of the target trait(s). S is determined by the intensity of selection (i), which is the number of genotypes selected relative to the total number under evaluation. Intensity of selection is the standardized selection differential:

$$i = \frac{S}{\sigma_P}$$

where σ_P is the square root of the phenotypic variance (Figure 2.4)

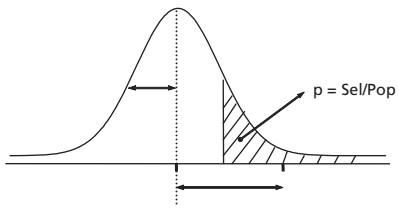
Alternative selection methods differ in the types of progenies evaluated and recombined, and the seasons required per cycle. Hence, Eberhart (1970) incorporated the number of years (y) and the parental control (c) into the prediction formula. After additional elaboration, a general expected genetic gain formula can be expressed as:

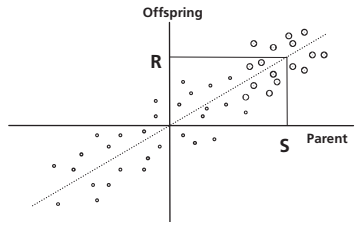
$$\Delta G = \frac{ic}{y}\frac{\sigma_G^2}{\sqrt{\sigma_P^2}} = \frac{ic}{y}\frac{\sigma_G^2}{\sqrt{\frac{\sigma_e^2}{re} + \frac{\sigma_{GE}^2}{e} + \sigma_G^2}}$$

where i = standardized selection differential, c = parental control (see below), y = seasons per cycle, r = number of replications per environment, e = number of environments, and σ_{Gl}^2, σ_{GE}^2 and σ_e^2 are as defined earlier. The variance components are estimated from the analysis of variance or mixed model solutions (Hallauer and Miranda, 1988; Holland, Nyquist and Cervantes-Martinez, 2003).

Increased heritability results in more effective selection. In the genetic gain formula, the value of the numerator can be increased by increasing the selection intensity, parental control or genetic variance. The value of the denominator can be decreased by decreasing the number of seasons per cycle or the phenotypic variance. Response to selection is greater when a lower proportion of individuals or families are selected. However, by decreasing the proportion selected (e.g. increasing selection intensity) the effective population size is reduced, thus increasing the possible occurrence of genetic drift or inbreeding. Desirable genes can be lost due to genetic drift. Because these effects can be detrimental for future gains, it is recommended to select a proportion of at least 20 percent. Parental control, c, can be increased by recombining genotypes where both sources of gametes originated in selected genotypes (c = 1). If the male gametes are coming from unselected genotypes, then c is 0.5. Hence, selection before pollination, where only selected genotypes contribute to the next generation, is more effective than selection after pollination, where non-selected geno-

FIGURE 2.4
Schematic representation of selection differential and response to selection





S = selection differential $S = x_{sel} - x_{pop} = i\sigma_p$

i = Standardized selection differential $i = S/\sigma_p = z/p$

$$R = b_{\bar{P}} S_o = h^2 S = i h^2 \sigma_p = \frac{i\sigma_g^2}{\sqrt{\sigma_p^2}} = \frac{c}{y}\frac{i\sigma_g^2}{\sqrt{\sigma_p^2}}$$

types can contribute to the next generation. Further increase of *c* can be accomplished by inter-mating selfed progenies of selected genotypes (*c* = 2). Genetic variance can be increased by selecting parents with high genetic diversity, increasing the degree of inbreeding before evaluation, increasing the recombination between cycles or using different types of progenies. Different types of progenies express—and therefore exploit—different proportion of genetic variances. The theoretical proportion of σ_A^2 of total σ_G^2 is 0.25 for half-sib progenies, 0.5 for full sib progenies and 1.0 for S_1 progenies. In addition to the type of progeny, different populations have different proportions of genetic variance components, and therefore different gains can be observed and expected.

The number of seasons required to complete a cycle can be reduced by using off-season nurseries at lower latitudes or in the opposite hemisphere, or by using greenhouses and growth chambers. The phenotypic variance can be decreased by increasing the number of replications and environments, or using improved statistical design and analysis techniques that reduce the error variance and more accurately estimate progeny performance. Increasing the number of environments affects selection response more than increasing the number of replications. However, the environments have to be representative of the target area because if the environments are very different, σ_{GE}^2 can increase substantially and thereby reduce selection gain. This is the case when, for example, the relative importance of stress factors differs between selection and target environment (Betrán, Bänziger and Menz, 2004; Cooper *et al.*, 2006).

Proper field experimental design and analysis (see also Chapters 3 and 20 in this volume), field stratification, number of plants measured, uniform soils and treatments, and reliable data collection and processing reduce the error term and subsequently increase the gain. Several field experimental designs are used in plant breeding. The most common are randomized complete block, incomplete lattices and, more recently, row-and-column designs among replicated designs, and augmented designs among unreplicated designs. Optimal experimental designs and field layout are useful in reducing the error, increasing the precision of mean estimates, and consequently improving selection (Gilmour, Cullis and Verbyla, 1997). Data processing

and analysis has improved in recent years, with more powerful computer hardware and software addressing G×E interaction of multi-environment trials, in-trial spatial variation, correlation among traits, variance components estimation, prediction of genetic parameters, QTL mapping, MAS, etc. (van Eeuwijk *et al.,* 2005; Romagosa, van Eeuwijk and Thomas, 2008).

The use of artificial inoculation with pathogens and infestation with major pests has increased display of genetic variation and the heritability and gain for host plant resistance. Similarly, the use of managed drought, nitrogen or low-pH stress environments has increased heritability and selection gain for abiotic stress tolerance (Bänziger and Cooper, 2001).

Recent developments in biotechnology mean that effectiveness of selection can be increased by the use of molecular tools. For example, molecular markers can be used to: (1) increase selection intensity while selecting genotypes with different genetic backgrounds to maintain genetic variance; (2) select before pollination genotypes with the desired allele composition for markers associated with traits of interest; (3) conduct selection in environments not representative of the target area (e.g. off-season nurseries); and (4) conduct MAS *per se* or in combination with phenotypic selection.

2.2.8 Correlated response to selection and indirect selection

Several relevant traits are often considered simultaneously in plant breeding, particularly when selection is done by farmers. The relationship among them determines breeding strategies and response to selection. The association among desirable traits can be negative (e.g. increasing grain yield is associated with lower protein content in maize (Duvick and Cassman, 1999)), or positive (e.g. reduced anthesis–silking interval under drought is associated with increased yield in maize (Edmeades, Bolaños and Chapman, 1997)). Correlations among traits can be due to pleiotropy (same loci affect both traits), linkage/linkage disequilibrium (different loci affect the traits but these loci are linked together), or environmental effects. Linkage in coupling will cause positive correlation and negative correlation in repulsion. If the environment affects both traits in the same way (e.g. plant height and grain yield in maize), it can create a positive correlation. Different types of correlations can be calculated: phenotypic correlations (r_P) are calculated using phenotypic values; genotypic correlations (r_G) are calculated using genotypic values; and additive genetic correlations (r_A) (also known as genetic correlations) are calculated using breeding values (Falconer and Mackay, 1996). Genetic correlations among traits can change with selection as a consequence of change in allelic frequencies.

If the direct selection response for trait X is $R_X = ih_X\sigma_{AX}$, the correlated response in trait Y is defined as $CR_Y = ih_X h_y r_A \sigma_{PY}$, where h_x and h_y are the square roots of heritabilities for traits X and Y, respectively; r_A is the genetic correlation between traits X and Y; i is the selection intensity; and σ_P is the phenotypic variance. Indirect selection for trait Y can improve trait X. Therefore the relative efficiency or merit of indirect selection can be compared with the direct selection for trait X as follows:

$$\frac{CR_X}{R_X} = \frac{i_Y h_Y r_A \sigma_{AX}}{i_X h_X \sigma_{AX}} = \frac{i_Y h_Y r_A}{i_X h_X}$$

(Falconer and Mackay, 1996)

Indirect selection can be more effective than direct selection when this ratio is >1 in cases where secondary traits show greater heritabilities than the primary trait

and high correlations between secondary and target traits are present, or if greater selection intensities can be applied to the secondary trait (e.g. easier to screen in big populations). If selection intensity in both traits were considered the same, indirect selection would be superior to direct selection when $r_A h_y$ is greater than h_x. Ideally, a secondary trait should be associated genetically with the target trait, highly heritable, easy and fast to measure, non-destructive, stable over the measurement period, and/or observable at or before flowering so that undesirable parents are not crossed (Edmeades, Bolaños and Chapman, 1997).

Selection indices combine information from different traits with the goal of selecting genotypes with the highest aggregate breeding value. Many breeders and especially farmers have an ideotype in mind when applying selection, which is a rather subjective application of a selection index. More objective selection indices are linear combinations of observable trait values that maximize the expected genetic gain in an aggregate breeding value. Baker (1986) and Lin (1978) have reviewed the theory of selection indices and their application to plant breeding.

MAS (i.e. selection of genotypes based on molecular markers associated with target traits) is also a form of indirect selection. MAS for quantitative traits can be used in situations where phenotypic selection can not be conducted (e.g. off-season nurseries), when target traits have low heritability and there are tight linkage between QTLs and markers, and a high proportion of additive variance is explained by the markers (Lande and Thompson, 1990; Hospital and Charcosset, 1997).

2.3 KEY EXPERIMENTS FOR RESPONSE TO SELECTION

Selection experiments have been designed to make the improvement of quantitative traits more efficient, trying to maximize as much as possible the additive effects, as well as to gather genes with complementary dominant and epistatic effects in genotype crosses and synthetic varieties. There are many examples that show the efficacy of these experiments. Below, we discuss the results of some selection experiments for complex traits published in the last years, which supposedly reveal the genetic architecture of quantitative traits. Experiments are divided into short-term, mid-term and long-term recurrent selection experiments. We are focusing this section on recurrent selection experiments because they reveal better than any other kind of selection method the role of the hidden genetic factors responsible for performance and behaviour of quantitative traits. Experiments that combine selection and subsequent recombination of selected genotypes permit the best exploration of the intricate assortment of both major genes and genes with small effect for the trait studied.

Recurrent or cyclic selection is generally applied to a population, where loci responsible for a quantitative trait are segregating with different gene effect and at different allele frequency, except for the case of an F_2 population derived from the cross of two inbred lines, in which allele frequencies for all segregating loci are 0.5. The aim of recurrent selection programmes is to increase progressively, cycle after cycle, the frequency of the favourable alleles responsible for the performance of the trait under selection (see later Chapters). The general scheme of each selection cycle involves three steps: (1) creation of a family structure from selection units; (2) evaluation

of familial test units in replicated trials in different environments, i.e. progeny testing; and (3) recombination of genotypes related to the families selected (recombination units) in the evaluation trial (Moreno-González and Cubero, 1993).

The above general scheme may be modified for different methods of selection, increasing or reducing the number of generations. For example, the three steps are combined in one generation per cycle in the recurrent mass selection method, and in two generations for the half-sib or full-sib family selection method as first introduced by Vilmorin in 1856 for sugar beet. In the case of mass selection, the selection units are individual plants, rather than families. In the case of half-sib and full-sib selection, creation of new families and recombination of selected families is the same operation, thus both steps are done in the same season, while family evaluation is carried out on the next generation. In contrast, any of the basic steps may involve more than one generation. For example, (1) creation of a family structure in the S_2 family selection method requires two generations; (2) evaluation trials may be conducted during more than one year either to reduce the effect of genotype × year interaction or to conduct selection in multiple stages, such as genotypes selected at low selection intensity in the first year may be re-evaluated at higher selection intensities in following years (Piper and Fehr, 1987); and (3) selected genotypes may be recombined during more than one generation to break up repulsion linkages among favourable alleles.

Many recurrent selection experiments have been reported during the last three or four decades in the literature, which show genetic improvement for the selected trait in several crops. We choose here to discuss some of these experiments based on three criteria: (1) enough information is available to rely on the accuracy of results concerning genetic improvement; (2) comparison among different selection methods is possible; and (3) additional information is provided to help us to interpret the structure of genetic and environmental factors involved in complex traits. Short-term, mid-term and long-term selections are arbitrarily designed here as those experiments that have undergone <6, between 6 and 20, and >20 selection cycles, respectively.

2.3.1 Short-term recurrent selection experiments. The case of the BS11 population

A maize population, BS11 from Iowa State University, USA, has been studied for several factors affecting the process of selection for complex traits in short-term recurrent selection experiments (Weyhrich, Lamkey and Hallauer, 1998a, b; Guzman and Lamkey, 1999, 2000). These authors focus on two useful concepts for plant breeders, which are worth comment: (1) comparisons of the response of the same population to different intra-population and inter-population methods of selection; and (2) the role of the effective population size in the selection response and the genetic variability of the population.

Six intra-population methods—full-sib, half-sib, mass, modified ear-to-row, S_1 progeny and S_2 progeny selections—and one inter-population method, reciprocal full-sib recurrent selection (RFSRS), were applied to the same BS11 maize population and compared for grain yield and other traits during five selection cycles, except for half-sib selection, for which only four cycles were completed (Weyhrich, Lamkey and Hallauer, 1998a). General descriptions of the above and other schemes of selection methods may be found in Moreno-

González and Cubero (1993) and in Chapters 5 to 13 in this volume. Averaged selection responses for the grain yield trait in the populations *per se* and the population testcrosses are summarized in Table 2.7. Improved populations were testcrossed to the original BS11 population and to inbred B79. The S_2 progeny selection method resulted in the highest genetic gain per cycle for both the population *per se* (4.5 percent relative to the original cycle) and the average over population testcrosses (3.3 percent), followed by the modified ear-to-row selection method, which showed a genetic gain of 3.6 and 2.7 percent for the population *per se* and the testcross average, respectively (Weyhrich, Lamkey and Hallauer, 1998a). The superiority of the S_2 progeny selection over the other methods in this experiment was attributed to the importance of additive effects relative to dominance effects in the BS11 population. In contrast, other reports had found that testcross or reciprocal selection methods were superior to inbred progeny methods, probably because the presence of non-additive relative to additive effects was important in those populations studied (Horner, Magloire and Morera, 1989; Lamkey, 1992; Holthaus and Lamkey, 1995a,b).

In addition, the S_1 progeny selection method was applied to the BS11 maize population during five cycles, using four different effective population sizes (i.e. recombination of 5, 10, 20 and 30 selected lines) but with a common selection intensity of 20 percent for all of them (Guzman and Lamkey, 2000). Comparison of the original population (C_0) and selection cycle five (C_5) showed that genetic gain for grain yield of C_5 relative to C_0 was significant for all effective population sizes, with the highest yield gain for effective population size 10, followed by population sizes 30, 5 and 20. No significant difference was found among the additive genetic variance and heritability estimates of the four C_5 effective population sizes and population C_0. The results of this study showed that (1) use of smaller population size would not limit genetic progress in short-term recurrent selection; and (2) there is no clear advantage to using a larger population size to maintain genetic variability in short-term selection experiments (Guzman and Lamkey, 2000).

2.3.2 Mid-term recurrent selection experiments

A summary of the genetic gains for several cycles of selection, selection methods

TABLE 2.7
Grain yield genetic gains (%) averaged over cycles for different recurrent selection methods in the maize population BS11

Selection method	Selection cycles (No.)	Genetic gain per cycle (%)	
		Population per se	Averaged over population testcrosses
Full-sib	5	1.4	1.6
Half-sib	4	1.6	2.1
Mass	5	0.6	0.5
Modified ear-to-row	5	3.6	2.7
RFSRS†	5	2.6	2.6
S1-progeny	5	1.9	1.6
S2-progeny	5	4.5	3.3

Notes: † = reciprocal full-sib recurrent selection.
Source: adapted from Weyhrich, Lamkey and Hallauer, 1998a.

TABLE 2.8
Genetic gains (%) averaged over cycles for different recurrent selection methods in the populations *per se* and population crosses, and the change in genetic variance through selection cycles

Crop and Trait	Selection method	Selected source population	Selection cycles (No.)	Genetic gain per cycle (%)		Change in genetic variance over cycles	References
				Population per se	Averaged population crosses		
Oat yield	$F_{4.6}$ lines	Univ. Minnesota	7	2.2		No change	De Koeyer and Stuthman, 1998
Spring wheat kernel weight	F_3 lines	Univ. North Dakota	8	4.5		No change	Wiersma *et al*., 2001
Maize yield	RFSRS†	BS10	5	3.2	2.5		Hallauer, 1984
	RFSRS	BS11	5	2.9	2.5		
Maize yield	RFSRS	BS10	7	2.0			Rodriguez and Hallauer, 1988
	RFSRS	BS11	7	0.8			
Maize yield	RFSRS	BS10	8	3.0	6.5		Eyherabide and Hallauer, 1991
	RFSRS	BS11	8	1.6	6.5		
Maize yield	RFSRS	BS10	10	2.7	1.6	No change	Frank and Hallauer, 1999
	RFSRS	BS11	10	2.3	1.6	No change	

NOTES: † RFSRS = reciprocal full-sib recurrent selection
Source: adapted from references.

and crops is shown in Table 2.8. Most of the experiments are for maize but two are reported for other crops.

Self-fertilized crop species

A recurrent selection programme to increase grain yield in oat has been carried out at the University of Minnesota, United States of America, since 1968. The selection method comprises three steps: (1) selection of 21 $F_{4.6}$ lines out of 630 tested; (2) creation of 63 F_1 single crosses by intercrossing the 21 selected lines, each with another six different lines; and (3) derivation of 10 lines from each F_4 (De Koeyer and Stuthman, 1998). The linear regression response to selection over seven cycles was 2.2 percent per cycle relative to the original population (Table 2.8). The results indicated that there has not been significant change in the estimates of the genetic variance through the seven selection cycles (De Koeyer and Stuthman, 1998).

A recurrent selection programme to increase kernel weight in spring wheat was initiated at Fargo, North Dakota, United States of America, in 1967, and moved to St. Paul, Minnesota, United States of America, after the fourth cycle (Wiersma *et al*., 2001). The selection scheme was similar to that described above for oat, but the number of evaluated and selected lines, the number of inter-crosses and the generation used for the derived lines were different. Kernel size increased linearly at 4.5 percent per cycle over eight cycles (Table 2.8), with an indirect increase in flour yield. Results indicated no clear trend towards a decrease in genetic variance. Results suggested that the trait is controlled by several genes with small effects (Wiersma *et al*., 2001).

The two above experiments are examples of the efficiency of recurrent selection for complex traits in self-fertilized crop species. Other reports of recurrent selection in autogamous plant species have also been published. Nine cycles of recurrent selection for groat-oil content in oat produced a linear increase in the groat (caryopsis) oil content of oat at a rate of 6.5 percent per cycle and non-decrease in the genetic variation (Frey and Holland, 1999). It seems that additive effects were predominant in

self-fertilized crops. Soybean and cotton populations have also undergone recurrent selections with similar schemes to those described above (Piper and Feher, 1987; Miller and Rawlings, 1967).

Inbred versus population tester in reciprocal recurrent selection experiments

Reciprocal recurrent selection (RRS), first designed by Comstock, Robinson and Harvey (1949), tries to alter two different genetically populations to improve their cross mean. The original method consists of the following steps: (1) individual plants from two populations, A and B, are selfed and at the same time crossed to 3 to 5 random plants from the reciprocal female tester population, B and A, respectively; (2) selection in each population is based on the performance of the testcross half-sib families; (3) remnant seed from the selected S_1 families are mated at random within A and B to form new cycles of the A and B populations. Russell and Eberhart (1975) proposed a modification of RRS (MRRS), suggesting to use as tester of population A an inbred line derived from or related to B, instead of the population B itself; reciprocally, the tester of B should be an inbred line derived from or related to A.

A programme to compare MRRS and RRS using the maize populations BS21 and BS22 was initiated in the maize breeding programme at Iowa State University in 1975 (Russell, Blackburn and Lamkey, 1992). After three cycles of selection, the populations *per se* and the population cross of the MRRS method showed less genetic response than populations selected under the RRS method (Russell, Blackburn and Lamkey, 1992). It should be specially noted that the improved population BS22(H99HI), which uses inbred H99 as tester in the MRRS method, had less genetic response, genetic variance and predicted genetic gain than the other populations involved in the study (Russell, Blackburn and Lamkey, 1992). Inbred A632 was the tester of selected population BS21(A632HI). It seems that the elite inbred H99 might have masked dominant, favourable alleles present in the BS22 population. Comstock (1979), using quantitative genetics theory, compared population improvement with both types of testers (i.e. inbred vs. population) based on change in allele frequency. He concluded that the inbred line tester was not superior to the reciprocal population tester. Furthermore, the population tester might be superior to the inbred tester in some situations, especially if overdominance and multiple peak epistasis are present in the populations. Likewise, Moreno-González and Grossman (1976) demonstrated that the theoretical genetic gain of the population cross was higher when a low-yielding population (i.e. smaller allele frequencies for the segregating loci) was used as population tester.

The expected change in allele frequency in the selected population (Δp_A) (Moreno-González and Grossman, 1976; Falconer, 1981) will be

$$\Delta p_A = \frac{i p_A (1 - p_A)\left[a + (1 - 2p_T)d\right]}{2\sigma_p}$$

where i is the selection intensity; p_A and p_T are the frequencies of more favourable allele in the selected and tester populations, respectively; a and d are the additive and dominance effects; and σ_p is the phenotypic standard deviation of testcross or half-sib families. The numerator of the above expression is expected to have the same value both when the tester is the reciprocal population B and when it is an inbred line randomly derived from B; however, σ_p is larger when the tester is an inbred line

(Comstock, 1979). Thus Δp_A is not expected to be higher for the inbred tester than for the reciprocal population tester.

The same populations, BS21 and BS22, and breeding selection methods, RRS and MRRS, were also evaluated after the sixth cycle (Menz Rademacher, Hallauer and Russell, 1999). Results from this study were essentially similar to that of Russell, Blackburn and Lamkey (1992) for cycle three. The grain yield response of the population cross, BS21 × BS22, was higher for RRS (4.4 percent per cycle) than for MRRS (1.6 percent per cycle), and also for the testcross direct responses of the MRRS method (2.8 and 1.6 percent per cycle for BS21 × A632 and BS22 × H99, respectively) (Menz Rademacher, Hallauer and Russell, 1999). It seems that the MRRS was less efficient than RRS for increasing the population cross BS21 × BS22. Efficiency of MRRS depends on the choice of the tester, which is related to the type of gene action involved in the complementary alleles between the tester and the selection populations. If a favourable allele with complete dominance is fixed in the inbred tester, frequency of this allele is not expected to increase in the selection population, whereas this frequency will increase if the allele is segregating in a population used as tester. Thus, a limitation exists in terms of increasing the frequency of dominant favourable alleles in the selection population when they are fixed in the inbred tester.

Reciprocal full-sib recurrent selection experiments

The RFSRS method was designed for maize yield selection by Hallauer and Eberhart (1970), and has been applied to the BS10 and BS11 maize populations at Iowa State University. This method has proven to be very efficient in increasing the genetic gain of both the populations *per se* and the population hybrid (Table 2.8; Hallauer, 1984; Rodriguez and Hallauer, 1988; Eyherabide and Hallauer, 1991; Frank and Hallauer, 1999). The direct selection response for grain yield per cycle in the cross between the two populations was significant for all reported studies of evaluation, but varied among the different studies, being 2.5, 6.5 and 1.6 percent per cycle when the first 5, 8 and 10 selection cycles were evaluated, respectively. Sampling of populations and different years and environments of evaluation might account for these differences. The indirect selection response of the populations *per se* was similar in the four studies reported (Table 2.8), and it was consistently higher in BS10 than in BS11. It seems that selection for the population hybrid increased the allele frequency of favourable alleles in BS10 itself at a higher rate than in BS11. No significant differences between cycle 0 and 10 were found for the grain yield genetic variances of the populations *per se* and the population hybrid (Frank and Hallauer, 1999). This suggests that further response to selection should be expected in the next cycles of RFSRS applied to BS10 and BS11.

Over fifty years of reciprocal recurrent selection experiments

A RRS programme has been conducted by the Cooperative Federal-State maize breeding programme at Iowa State University with the synthetic maize populations BSSS and BSCB1 since 1949 (Keeratinijakal and Lamkey, 1993a), the year when the RRS method was first published (Comstock, Robinson and Harvey, 1949). Several reports on the genetic improvement in grain yield and other traits have been published through all the history of the RRS programme (Eberhart, Debela and Hallauer, 1973; Smith, 1983; Oyervides-

TABLE 2.9
Average genetic gains (%) of grain yield over cycles for the reciprocal recurrent selection (RRS) method applied to the maize populations BSSS and BSCB1 in the populations per se, population cross and population topcrosses

Selected population	Cycle No.	Genetic gain per cycle (%)			Change in genetic variances	References
		Population *per se*	Population cross	Population topcross		
BSSS	5	-0.1	4.1	1.2		Eberhart, Debela and Hallauer, 1973
BSCB1	5	1.0	4.1	0.3		
BSSS	7	2.2	4.3			Smith, 1983
BSCB1	7	0.7	4.3			
BSSS	8	1.9		1.4		Oyervides-Garcia and Hallauer, 1986
BSSS	10	1.3				Rodriguez and Hallauer, 1988
BSCB1	10	-1.6				
BSSS	9		6.1		Increase in additive and no change in dominance genetic variances of population cross	Betrán and Hallauer, 1996a, b)
BSCB1	9		6.1			
BSSS	11	1.7	6.9	2.8		Keeratinijakal and Lamkey, 1993a
BSCB1	11	1.9	6.9	3.9		
BSSS	11	2.6			No change in additive and reduction in dominance variance	Holthaus and Lamkey, 1995a

Source: adapted from references.

Garcia and Hallauer 1986; Rodriguez and Hallauer, 1988; Helms, Hallauer and Smith, 1989; Keeratinijakal and Lamkey, 1993a,b; Holthaus and Lamkey, 1995a,b; Betrán and Hallauer, 1996a,b). Some of the results for grain yield are summarized in Table 2.9. The direct response to selection in the population cross was reported to be very effective in four independent studies, 4.1 percent per cycle (Penny and Eberhart, 1971), 4.3 percent per cycle (Smith, 1983), 6.1 percent per cycle (Betrán and Hallauer, 1996a) and 6.9 percent per cycle (Keeratinijakal and Lamkey, 1993a), when the first 5, 7, 9 and 11 selection cycles were evaluated, respectively. However the observed indirect response to selection was much smaller in the populations *per se* than in the population cross, ranging from -1.6 percent per cycle in BSCB1 (Rodrigez and Hallauer, 1988) to 2.6 percent per cycle in BSSS (Holthaus and Lamkey, 1995a).

Part of the lower selection response in the populations *per se* compared to the population cross can be attributed to the genetic drift caused by restricted effective population size during the process of selection. The inbreeding coefficient progressively increases in the populations *per se* when the number of selected lines used for recombination has been small (i.e. <20) during several selection cycles. The expected inbreeding coefficient of a diploid population reproduced with a finite number of selected individuals during t generations can be estimated as:

$$F_t = 1 - (1 - F_0)\prod_{i=1}^{i=t}(1 - \frac{1}{2N_i})$$

where F_0 and F_t are the inbreeding coefficients of the original population and after t generations of selection, respectively; and N_i is the number of individuals selected at generation i.

If $F_0 = 0$, and N_i is constant (i.e. N) for all generations, the above equation becomes the known formula:

$$F_t = 1 - (1 - \frac{1}{2N})^t$$

In this case, if $N = 10$ and $t = 14$, then $F_{14} = 0.512$, which is larger than after one generation of selfing. Thus, inbreeding depression may become evident in these selected populations if dominance effects controlling the trait under selection (i.e. grain yield) were important. When responses were adjusted for effects of genetic drift, the improvements of the populations *per se* were similar to those of the population cross in the study of Keeratinijakal and Lamkey (1993b), and larger than in Smith's study (1983). In addition, additive and dominance effects were found in BSSS, but only dominance effects were important in BSCB1. The presence of important dominance effects may explain the inbreeding depression of the populations *per se*.

For grain yield, the additive genetic variance of population BSSS did not decrease after 11 cycles of RRS, whereas the dominance genetic variance was reduced, and the heritability estimates increased in BSSS when cycle 11 was compared to cycle 0 (Holtaus and Lamkey, 1995a). Likewise for grain yield, the additive genetic variance of the population cross BSSS × BSCB1 increased after nine cycles of RRS, with no reduction in the dominance genetic variance, and an increase in the heritability estimates (Betrán and Hallauer, 1996a). Therefore, it seems that the response of these populations to RRS will continue in the next cycles of selection.

Restriction fragment length polymorphism (RFLP) loci were used to determine changes in allele frequency, expected heterozygosity, and genetic variation in the populations BSSS and BSCB1 after 12 cycles of RRS (Labate, Lamkey and Woodman, 1999). Allele frequency changes in 28 loci, out of 82, could not be explained by genetic drift, thus it should be attributed to selection. The within-population expected herezogosity decreased, while the inter-population component of genetic variation increased (Labate, Lamkey and Woodman, 1999). Also, another study was conducted to investigate the genetic variation of progenitor inbred lines used to synthesize populations BSSS and BSCB1, as well as elite inbred lines derived from advanced cycles of selection (Hagdorn *et al*., 2003). A larger genetic distance was found between the BSSS and BSCB1 groups of lines derived from advanced cycles than between the group of lines derived from cycle 0. Thus, these studies confirm the success of RRS in increasing the genetic diversity of the two populations, which was one of the objectives of the method. Also, these results reinforce the hypothesis of the complementary effects of heterozygous genes in the population cross, and the hybrids between elite inbred lines derived from the two reciprocal populations.

2.3.3 Long-term recurrent selection experiments

Mass selection experiment for ear length in maize

A mass selection programme started at Iowa State University in 1963 to select for divergent ear length (i.e. short and long ears) in the maize population BSLE (López-Reynoso and Hallauer, 1998). A modified scheme of mass selection proposed by Gardner (1961), called grid selection, was used in this experiment. The scheme subdivides the selection field into 100 plots, each with 40 plants, among which three plants per plot (i.e. 7.5 percent selection

pressure) were selected for long and short ear. Each selection cycle was completed in one year. The linear selection response was 1.4 percent per cycle for longer ears, and 1.9 percent per cycle for shorter ears over 27 selection cycles. The rate of the inbreeding depression in selfed populations relative to unselfed was about 18 percent for longer ears and remained constant during the process of selection, whereas this rate gradually reduced for shorter ears as generations advanced, from 18.2 percent in the original population up to –2.9 percent in the 24th cycle (López-Reynoso and Hallauer, 1998). If the genetic effects of some loci are assumed to be dominant for longer ears, then the recessive alleles of these loci would be more easily fixed for shorter ears in the course of selection. Thus, no alleles with dominant effects would be segregating in the selected population for short ears after cycle 24, which would explain the absence of inbreeding depression in the short-ear population in comparison with the long-ear population. Genetic variance for ear length still remained in both populations after selection cycle 24, although the heritability of the trait was reduced.

Mass selection experiment for prolificacy in maize

A mass selection programme was initiated at the University of Wisconsin-Madison in 1971 to select for prolificacy (i.e. increase in number of ears per plant) in the maize open-pollinated population GG(MP) (de Leon and Coors, 2002). The selection response of the programme was an increase of 0.14 and 0.03 ears per plant per cycle at low and high plant densities (15 000 and 73 000 plants/ha), respectively, during 24 selection cycles. Most of the response was achieved between cycles 18 and 24. Indirect responses were also sensitive to selection. An increase in number of ears per plant brought a decrease in other ear traits affecting yield, such as ear length, ear diameter and kernel size, revealing that grain yield is a very complex trait and individual selection for one of the yield component does not necessarily have a significant effect on the yield trait.

Over a century of an Illinois long-term selection experiment for oil and protein content in the maize kernel

The Illinois long-term selection experiment begun in 1896, analysing percentage oil and protein in 163 ears of the maize open-pollinated variety 'Burr's White' (Dudley, 1977). The 24 ears highest in protein, the 12 ears lowest in protein, the 24 ears highest in oil, and the 12 ears lowest in oil were selected to initiate the Illinois high protein (IHP), Illinois low protein (ILP), Illinois high oil (IHO) and Illinois low oil (ILO) strains, respectively (Dudley and Lambert, 1992). Results and studies of this experiment have been published in many reports (Leng, 1962; Dudley and Lambert, 1969; Dudley, Lambert and Alexander, 1974; Moreno-González, Dudley and Lambert, 1975; Dudley, 1977; Dudley, Lambert and de la Roche, 1977; Dudley and Lambert, 1992; Dudley, 1994; Sughroue and Rochefort, 1994; Moose, Dudley and Rochefort, 2004; Laurie *et al*., 2004; Dudley and Lambert, 2004; Clark *et al*., 2006; Dudley, 2007, 2008). So far, 106 cycles of recurrent selection have been carried out on these strains (Dudley, 2007). The schemes of the selection methods were slightly modified through generations. Mass selection was used during generations 0–9; ear-to row during generations 10–25; and half-sib selection for percent oil and mass selection for percent protein during generations 26 to date (Dudley, 1977; Dudley and Lambert, 1992).

This experiment reveals the power of long-term recurrent selection for achieving progressively significant genetic changes in quantitative traits, such as protein and kernel oil content, through more than one hundred selection generations. Responses to selection for the high and low strains of oil and protein in the maize kernel were very important in the long run. Populations changed from 4.6 percent oil to 19.6 percent in IHO, and to 0.5 percent in ILO after 87 generations. At generation 89, selection for ILO was discontinued because a biological limit had been reached in terms of maintaining seed viability of the ILO strain (Dudley, Lambert and Alexander, 1974; Dudley and Lambert, 1992). For protein, populations changed from 10.9 percent to 32.5 percent in IHP and to 4.2 percent in ILP after 90 generations. Progress in the ILO and ILP lines had ceased in the latter generations (Dudley, Lambert and Alexander, 1974; Dudley and Lambert, 1992). In terms of additive genetic standard deviations (σ_a) of the populations, the genetic gains became huge, being 22 σ_a for IHO and 26 σ_a for IHP (Dudley and Lambert, 1992). These deviations are placed in the extreme tail of the normal distributions, with probabilities less than 10^{-105} and 10^{-145} for 22 σ_a and 26 σ_a, respectively. Thus, assuming that recombination of favourable loci could be gathered at random in a unique genotype in one generation, it would be necessary to grow an unimaginable number of maize plants (10^{105} and 10^{145} plants) to find one plant with the same oil and protein percentages as IHO and IHP, respectively. If no more than 10^{14} maize plants are currently grown in the world every year, it would be necessary to wait for at least 10^{91} and 10^{131} years to find at random the IHO and IHP strains that have been selected in only 100 years by applying simple recurrent selection methods. Genetic variance still remains in the IHO and IHP strains, because selection progress continued at the same rate in the two strains from generations 76 to 90. In addition, significant genetic variances were directly estimated in the strains in the 65th generation (Dudley and Lambert, 1969).

Besides the potential breeding benefit of developing maize strains for high and low oil and protein, this experiment has been a good tool to look into the intricate complexity of the genetic architecture of a quantitative trait. The experiment has also been a test bench to check quantitative theory and to estimate some genetic parameters otherwise difficult to compute. The question that arises is: What we can learn from this long-term selection experiment?

1. Recurrent selection was able to achieve a steady increase in the selection traits over more than one hundred generations. Mild selection (20 percent selection pressure) and adequate effective population size (>20) should have had a favourable effect in achieving this selection progress. The experiment has been a text-book example of selection. Recombination of selected genotypes created new genotypes distributed around new displaced means generation after generation. Thus, recombination and selection were the key points for reaching this huge genetic gain (i.e. 22 σ_a and 26 σ_a), which otherwise would not be possible to attain.
2. The genetic variance is not still exhausted. Reverse selection strains (i.e. RHO, RLO, RHP, and RLP) and switch-back selection from the reverse strain (SHO) clearly indicate that loci are still segregating in the populations and selection can be conducted in the desired direction at the same or higher rate as before.

3. By applying quantitative genetics theory to the genetic gains of the divergent selection strains, Dudley (1977) was able to estimate 54 loci for oil and 122 for protein, as well as the average allele frequency of alleles controlling the traits in the initial populations. Considering that oil and protein traits are not as complex as the yield trait in most of crop species, the number of loci responsible for yield should probably be higher.
4. Linkage disequilibrium in the coupling phase was detected for oil (Moreno-González, Dudley and Lambert, 1975) and protein (Dudley, 1994) using a Design III (DIII) of North Carolina in the F_2 and F_6 generations. This DIII was also able to estimate significant additive and dominance variances in the cross population. Reduction of additive variances from the F_2 to the F_6 generation suggested that loci for low and high oil and protein were coupled. For oil, there was no reduction in the dominance variance from the F_2 to the F_6 generation, suggesting that loci with dominance for low oil are combined with loci having dominance for high oil.
5. Single nucleotide polymorphism (SNP) molecular markers were used in the cross population of IHO × ILO, followed by 10 generations of random mating and one of selfing to look for associated QTLs (Laurie *et al.*, 2004; Clark *et al.*, 2006; Dudley, 2007). Molecular marker results confirmed the estimates of genetic parameters and the hypothesis proposed on the basis of quantitative genetics theory several years before. The number of QTLs estimated for oil was about 50, which was similar to the number of loci estimated by Dudley (1977). QTLs had small additive and dominance effects, which is congruent with the steady and continuous progress throughout generations and with no apparent exhaustion of genetic variance in the selected strains. Most of the QTLs with additive effects were in coupling phase, but some of them were also in repulsion, in agreement with the DIII studies (Moreno-González, Dudley and Lambert, 1975; Dudley, 1994). Breaking up the repulsion phase linkage would further increase selection response. QTLs had dominance effects for both high and low oil, as suggested by Moreno-González, Dudley and Lambert (1975).

2.3.5 Conclusions of empirical response to selection

A large body of evidence has been accumulated through selection experiments indicating that quantitative genetics is a useful empirical tool to model responses to selection. The genetic architecture of complex traits conforms to the infinitesimal hypothesis that postulates many genes segregating in the populations, with small additive and dominance effects and different allele frequencies. Also a few genes may have larger effects, and dominance effects may be present for both favourable and unfavourable alleles. Recurrent selection has been, is, and will be in the future, an effective strategy for improving complex traits in the desired direction. Choice of the selection method depends on the prevalence of additive or dominance effects. Intra-population selection methods mainly accumulate additive effects, while inter-population methods favour the presence of heterozygosity, differences in allele frequencies and the presence of dominance effects in the population cross. Genetic variance generally was not depleted in the selected populations, even in long-term

selection experiments. Likewise, heritability estimates were frequently no lower in the most advanced selected populations. Either the favourable alleles are hard to fix, or mutant alleles with small effects may naturally arise in the populations and subsequently may be captured during the selection process. Small effective population size is not an impediment for short-term selection, but it might limit selection in the long term. Inbreeding depression of selected population may be caused by small population size during the process of selection in traits where dominant favourable alleles are important. The inbreeding depression disappears when the selected populations are crossed to others.

Epistasis is well documented and has been recognized as a not rare phenomenon in Mendelian qualitative traits that are controlled by two or three loci. Thus, epistasis should be also expected in more complex traits. Analysis of generation means has been frequently used to look for epistasis in quantitative traits. The magnitude of epistasis effects detected was small relative to the additive and dominance effects in most of the studies, although some traits and elite crosses showed up significant epistasis (Moreno-González and Dudley, 1981; Melchinger, 1987; Melchinger, Schmidt and Geiger, 1988; Lamkey, Schnicker and Melchinger, 1995; Hinze and Lamkey, 2003). Either the favourable and unfavourable epistatic effects involving the trait cancel out through the genome, or the genetic models or the statistical methods used are not powerful enough to separate epistasis from other effects. Dudley (2008) analyzed 500 S_2 lines derived from the crosses IHO × ILO and IHP × ILP, using SNP molecular markers. He reported that epistasis could contribute to the long continued response to selection in the Illinois long-term selection strains, and also may help explain the continued success of commercial corn breeding.

2.4 SUMMARY AND CONCLUSIONS

We have described the evolution of selection and plant breeding theory, the conceptual basis of key components of plant breeding approaches, and empirical examples of selection. The contribution of plant breeding to the improvement of crop production and quality has been enormous. Critical in this contribution has been the implementation of more efficient breeding approaches supported by developments in quantitative genetics. Plant breeding has evolved and will continue to evolve, adopting classical and modern technologies (e.g. family selection, recurrent selection, multi-location evaluation, off-season nurseries, biotechnology) to increase efficiency of selection, to adjust to new environmental conditions and variable demands for crop utilization, and to implement sustainable production systems. Plant breeders and geneticists will continue the search for and adoption of the more effective methods to develop, identify and evaluate cultivars that can contribute to superior agronomic performance and sustainable profit.

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CHAPTER 3

Main stages of a plant breeding programme

Salvatore Ceccarelli

3.1 INTRODUCTION

This Chapter describes the main stages of a plant breeding programme, which represent the technical aspects of the process through which new varieties are generated. The organizational aspects will be dealt with in Chapter 9.

Schnell (1982) described three main stages of a plant breeding programme, namely:

1. ***Generating genetic variability.*** This includes:
   - making crosses (selection of parents, crossing techniques, type of crosses);
   - induced mutation; and
   - introduction of germplasm.
2. ***Selection,*** i.e. utilization of the genetic variability created in the first stage. This includes primarily the implementation of various breeding methods, such as classical pedigree, bulk-pedigree, backcross, hybrids, recurrent selection, F_2 progeny method (in self-pollinated crops), synthetic varieties and hybrids (in cross-pollinated crops), and clones and segregating populations (in vegetatively propagated crops).
3. ***Testing of experimental cultivars.*** This includes comparison between existing cultivars and the breeding materials emerging from stage 2, and the appropriate methodologies to conduct such comparisons.

There are two other important stages in a breeding programme: setting priorities; and dissemination of cultivars. These two steps are considered in Chapters 4 and 21, respectively.

Before describing the essential features of the various stages, it is important to underline that plant breeding is a cyclic process in which each step feeds information into the subsequent step, and each cycle feeds information into the next cycle. Therefore a major challenge in a breeding programme is how to capture the information that is generated, in a way that is sufficiently transparent for others (scientists and non-professionals) to use.

In conventional breeding programmes, most of this information represents the 'cumulative experience' or the 'knowledge of the germplasm' that the breeder slowly accumulates over the years. In a participatory programme, it is important to maintain the typical cyclic character of plant breeding to ensure that all the participants have the possibility of accumulating and sharing the information generated in each step and in each cycle.

3.2 GENERATING GENETIC VARIABILITY

3.2.1 Crosses

The most common way of generating variability across crops with different mating systems is to make artificial and deliberate crosses among parents selected for specific traits (see also Section 6.3 in Chapter 6) with the objective of combining them in at least a fraction of the progenies. Suitable parents can be received from other breeding programmes, or are extracted from germplasm collections after searching the passport data for specific attributes, or are successful cultivars, including the most commonly grown cultivars or landraces. Wild relatives (mostly in the case of rice, wheat and barley) are also used, although not by all breeding programmes and not routinely, as sources of genetic variation not available in the corresponding crop. The generalized use of wild relatives in breeding programmes is restricted in several cases by crossing barriers with the corresponding cultivated crop.

Because of the cyclic nature of a breeding programme, the majority of parents in any given cycle are represented by the best lines selected from the previous cycle.

Parental material for specific traits can also be identified through specific screening activities. A typical example is resistance to biotic stresses, for which a routine parallel activity in a breeding programme is to screen germplasm either under artificial inoculation or in 'hot spots', i.e. in a location with a very high incidence of natural infection or pest challenge. Another example is the case of quality characteristics, in which germplasm is screened for the presence or amount, or both, of specific compounds associated with a given food, feed or processing quality.

Parental material can also be characterized for the adaptation to different environments or countries, and therefore crosses can be made with the objective of producing breeding material with general adaptation to particular environments or countries. Examples are screening under controlled conditions for resistance to salinity, cold, drought, or for micro-elements such as boron, manganese or aluminium (Marshall, 1991; Karakousis *et al*., 2003).

Backcrossing—even though it is often described as a breeding method—actually refers to a type of cross to generate variability in a fashion that increases the frequency of desirable combination of traits, i.e. most of the traits from the recurrent parent and one or a few from the non-recurrent parent.

A number of molecular tools are available today to assist the breeder in the selection of parents and in subsequent stages, and they are described in Section 19.5 (Chapter 19).

The number of crosses made by a breeding programme depends on various factors, such as the number of different objectives, the resources available and the breeding method used, which affects the number of breeding lines generated. In general, a breeding programme with a regional perspective (i.e. serving a single country or state) performs between 150 and 200 crosses per season on average. The number can go up to several thousand in international breeding programmes. On the issue of the number of parents, Witcombe and Virk (2001) have proposed the use of a low number of crosses (see also Section 6.5.2 (Chapter 6) for further details on their theory).

When the objective of the breeding programme is to produce hybrids, the variability is generated through methods of population improvement based on recurrent selection or through crosses between elite inbred lines (see Section 11.5 in Chapter 11 for further details).

In a number of cross-pollinated species, hybrid vigour is exploited by developing synthetic varieties through intercrossing a number of genotypes of known superior combining ability, i.e. genotypes that are known to give superior hybrid performance when crossed in all combinations. In contrast to hybrids, the seed of synthetic varieties can be used for succeeding seasons, and for this reason synthetic varieties are common in crops, such as forage crops, where cost precludes the development or use of hybrid varieties.

3.2.2 Exploiting existing variability

It is widely recognized that landraces and wild relatives harbour large amounts of genetic variability, which, in the case of self-pollinated crops, is readily available, as landraces and wild relatives are largely composed of different homozygotes. In these cases, the first step is represented by the collection of single spikes or plants, while their evaluation represents the second stage of the breeding programme.

3.2.3 Mutation

The use of artificially induced mutations, described in detail in Chapter 8, dates back to the origin of plant breeding as one of the ways to generate genetic variability beyond and besides that available naturally. The reader is referred to that Chapter for the technical aspects of induced mutations in plant breeding.

3.2.4 Generating genetic variability in a participatory breeding programme

In a truly participatory breeding programme, i.e. a programme that maintains the cyclic nature of a breeding programme, it is not extremely important who generates variability by, for example, making crosses, in order to categorize the type of participation. In fact, in any breeding programme, the degree of participation is determined by who selects the parental material. In a participatory breeding programme, if the farmers participate in the selection, they implicitly participate in the choice of the parents even if they do not physically make the crosses.

In a participatory programme, farmers can also contribute to the first step by suggesting the type of germplasm that is more likely to be acceptable in a given area.

3.3 SELECTION

The main characteristic of the selection stage is the utilization and narrowing down of the variability generated in the first stage and includes various steps by which the large diversity of breeding material (genetically of different types, depending on the mating system of the crop) is reduced to a number of lines suitable for the third stage.

The second stage is critical because the choices that are made during the various steps depend on the genetic control of the trait(s) under selection and on the environment in which the decisions are taken. Selection environments should be chosen to jointly reproduce the response of materials over the target region, or to be representative of the target environments of different agro-ecological subregions when breeding distinct varieties for each subregion (see Chapters 9 and 20).

The methods on how to handle the breeding material during the second stage vary considerably depending on the mating system of the crop, and these are dealt with in the respective chapters.

3.3.1 Self-pollinated crops

In self-pollinated crops, the products of the first stage are known as 'segregating populations', and the most common methods of handling them are described in Chapter 10. All the methods have in common a progressive increase in homozygosity, a reduction in the genetic variance within families, and an increase in the genetic variance between families.

In conventional breeding programmes, all the steps of stage 2 take place typically within a research station. In the initial steps of this stage the amount of breeding material is very large (often several thousand entries), and it is hard to organize their evaluation outside a research station. However, some breeding methods are particularly suited to the evaluation of the early segregating populations in the target environment (see Chapters 9 and 22), and enable the participation of farmers already at such an early stage. For example, in the bulk-pedigree method, the segregating populations, usually F_2 or F_3, each derived from a different cross, can be tested in a number of locations using an experimental layout similar to the yield trials (see stage 3), with the number of locations dependent on the diversity of the target population of environments and on the amount of seed available. In such cases, the

selection can be practised between crosses in the target environment, and within crosses on station for traits with high heritability.

Single-seed descent (described in Chapter 10) is one of the best ways of exploiting the variability within a superior cross and is fully compatible with a participatory programme as long as farmers contribute to the identification of the superior cross.

3.3.2 Cross-pollinated crops

The methods of exploiting the variability generated in the first stage in cross-pollinated crops vary according to the final product that the programme aims to produce (a hybrid, an open-pollinated variety, a synthetic variety, etc.).

When the objective of the breeding programme is to produce hybrids, the variability generated through methods of population improvement is exploited through the development of inbred lines (the methods to do that are described in Chapter 11), which are then crossed to produce the commercial hybrids. In the case of hybrid production, farmers could be involved both in the second step by contributing to the evaluation of the inbred lines, and in the third step by participating in the evaluation of the hybrids.

3.4 TESTING OF EXPERIMENTAL CULTIVARS

Testing of potential cultivars is the last stage of a breeding programme, which eventually ends with a new variety recommended for cultivation.

Usually, this stage takes place partly on research stations and partly in farmers' fields. However, there are exceptions, the best known being the breeding programmes in Australia where all the yield testing actually takes place in farmers' fields.

In most of the cases where the yield testing is conducted partly on station and partly in farmers' fields, the testing on research stations usually covers a period of three years. Most commonly, the number of breeding lines entering the testing stage is progressively reduced by discarding those that performed below a given standard. The most commonly used agronomic trait used to promote or discard breeding material during the testing stage of a breeding programme is grain yield. However, when the objectives of a breeding programme include, for example, quality characteristics and disease resistance, traits such as seed size or reaction to diseases complement grain yield.

The testing of experimental cultivars has a number of methodological and philosophical issues. The methodological issues include field plot techniques, design of trials (replicated vs. unreplicated trials), analysis of variety trials, and the organization and structure of Multi-Environment Trials (METs). The philosophical issue is primarily whether the testing of experimental cultivars should be conducted in an optimum climatic and agronomic environment or should be conducted in the target environment. When selection is conducted in the target environment, the breeding programme has to decide how many and which target environments to serve (Chapter 20).

One of the basic principles to apply in implementing the third stage of a plant breeding programme is that *field trials are expensive, and therefore the breeder should always find an optimal compromise between the number and the size of the trials, their precision, and the amount and the relevance of the information generated.* An ideal system of testing of experimental cultivars is a system that has the capacity to self-monitor its efficiency, effectiveness and relevance, and has flexibility to allow changes.

3.5 EFFICIENT EXPERIMENTAL PROCEDURES

Field plot techniques, as well as the choice of efficient experimental designs, aim at reducing the experimental error, thereby increasing the heritability and increasing the response to selection. Adopting efficient experimental procedures has paramount importance not only in the selection stage, but also in the testing stage of a breeding programme.

3.5.1 Field plot techniques

It is commonly believed that error variances tend to be larger under stress than under non-stress conditions, and this belief is a common justification for breeders to do most of the work on station or under optimum conditions. Even though this belief is hardly supported by experimental evidence (Al Yassin *et al*., 2005; Comadran *et al*., 2008), and because at the moment of planting it is difficult to predict how uniform a particular piece of land is going to be, it is always safer to put in place a set of measures to control at least some predictable sources of experimental error.

When genotypes are compared at increasing levels of moisture stress, small variations in soil depth and texture have increasingly large effects on plot-to-plot variability. Under these conditions, competition among genotypes for water also increases and bordering becomes critical (Fischer, 1981). For example, the yield of the outside row as a percentage of the yield of well-bordered rows in a maize nursery increased from 124 percent to 185 percent as yield levels were reduced by drought from 5 to 1 t/ha (Edmeades, pers. comm.). Many breeding programmes assume that all plots are equally affected by the border effect, and do not remove plants bordering alleys prior to harvest. However, this can introduce significant error in yield estimates, as there is strong genotype × border effect interaction. In fact, the decline in plant height from the edge to the centre of the plot can be used when selecting stress-tolerant lines (Rosenow, 1987; Blum, 1988). Reduction of border effects can be achieved very effectively in small-grain cereals by avoiding empty rows between adjacent plots and by planting the alleys. The resulting 'dirty' alleys are not very attractive, but their effect on uniformity within the plots is remarkable. Removing the alleys can be done at heading or shortly before maturity. This technique is particularly useful when testing is done in farmers' fields because, as mentioned in Chapter 9, farmers do not like to leave land empty on their property.

The control of border effects is also important in breeding nurseries, usually planted as individual rows or as two-row plots. The common practice is to leave one empty row between adjacent entries. The result is that everything we observe is border effect—with the exception, perhaps, of simply inherited characters.

Small plots should be avoided as much as possible when conducting yield trials. Table 3.1 shows an example of the effect of plot size on selection efficiency in barley on a dry site.

Competition among progenies grown in single- or double-row plots may lead to the identification of genotypes that owe their yield superiority only to the lack of aggressive rooting or smaller plant height of their neighbours. These advantages are nullified when the selected 'superior' cultivar is grown in pure stand. Constraints on seed per progeny and cost of labour and land often make additional bordering difficult.

Missing rows also have a marked effect on the performance of neighbouring rows, and it is advisable to check lines for germi-

TABLE 3.1
Efficiency of selection for grain yield as affected by plot size during the testing stage

Selection criterion in the previous season	No. of lines	Plot size	No. and percentage of lines outyielding the best local check	
			Dry site	Wet site
Grain yield	13	2 rows	5 (38.5%)	1 (7.7%)
Grain yield	21	4 rows	17 (81.0%)	2 (9.5%)

TABLE 3.2
Average yield (kg/ha) and coefficient of variation in barley yield trials conducted in two locations in northern Syrian Arab Republic as lattice designs

Trial No.	Tel Hadya (352.6 mm rainfall)		Bouider (243.6 mm rainfall)	
	Mean	c.v.	Mean	c.v
1	3715	17.7	1006	11.6
2	3337	12.8	777	15.8
3	3290	10.2	808	12.3
4	3005	15.3	955	12.6
5	2759	15.1	923	14.9
6	3195	12.1	1025	14.1
7	2993	13.7	1029	8.2
8	3156	15.0	997	13.1
9	3271	15.4	1031	11.0

nation prior to establishing a trial on a dry site if there is reason to believe that some entries will not germinate completely.

A series of check entries, spaced at regular intervals throughout unreplicated progeny trials, is essential to compensate for the effects of soil variability. Plot data are expressed relative to the performance of the check, adjusted for the physical distance between the nearest check plots and the plot in question. The check genotypes must always include the farmers' cultivar(s), lines that are well known to the breeder, and the best lines previously identified by the breeding programme. A progeny trial should be arranged in the field to ensure that check entries will not all be in the same columns; rather, they should form a grid which will provide a visual impression of the uniformity (or the variability) of the field.

When all these techniques are used on sites with low yield potential due, for example, to moisture stress, environmental variability can be kept at levels comparable with those of well-managed research stations with high average yields. As an example of what could be achieved by the package of plot techniques described in this section and the use of the experimental designs described in the next section in controlling environmental variability, we compared the coefficient of variation of nine trials grown on a stress site (average yield = 0.95 t/ha) and on a non-stress site (average yield = 3.19 t/ha) (Table 3.2). At yield levels where breeders usually do not work because the coefficient of variation is too large, the lattice design was capable, in most of the trials, of keeping it within acceptable limits.

A common source of error in conducting yield trials is an uneven plot length resulting from trimming plots to eliminate the edge effect. An effective way of ensuring a uniform plot length is to spray a herbicide using booms placed on a rigid arm

at a distance equivalent to the desired plot length.

3.5.2 Design of trials

The choice of an appropriate experimental design is another important decision affecting the precision of a trial.

One of the first issues in discussing the design of a trial is the alternative of replicated vs. unreplicated trials. In the testing stage of a breeding programme, which typically goes through three steps, the number of breeding lines being tested is usually in the range of several hundreds or even a few thousand in the first step, reducing to usually less than 50 in the third.

The use of replicated designs with several hundred or a few thousand lines implies the use of large areas, which makes the control of experimental error more problematic. In this step the number of experimental units (plots) per breeding line is limited by the amount of seed, which is usually small at this stage.

The combination of limited amounts of seed and of a large number of lines has made popular the use of unreplicated designs with systematic checks and with a row and column arrangement of the plot, which makes it possible to use spatial analysis (Singh *et al*., 2003).

One improvement over the unreplicated design with systematic checks is a partially replicated design where only a certain percentage of entries is replicated while still maintaining the systematic (also called grid) checks. In addition to the expected higher precision, the design responds to a frequent problem in the initial stages of testing, that is the different amount of seed available for different entries.

In replicated yield trials, where genotypes under test may number 200–500, improved statistical designs can lead to important increases in trial efficiency.

Unfortunately, despite the greater efficiency of lattice designs, of generalized lattices (Patterson and Hunter, 1983) and of neighbour analysis (Cullis and Gleeson, 1989), the randomized complete block design is still dominant in many breeding programmes in developing countries, particularly in those conditions where an increase in trial efficiency is most needed.

The use of generalized lattice designs (Patterson and Williams, 1976) combines good error control with flexibility in the numbers of treatments required. A promising extension of this design that removes both row and column effects has been described by Patterson and Robinson (1989). Nearest-neighbour analysis (e.g. Wilkinson *et al*., 1983; Hinz, 1987) have been used extensively in Australia to remove the effects of gradients of moisture stress within replicated and unreplicated trials (Marshall, 1987).

Spatial variability is a reality in field trials and a proportion of this is accounted for as inter-block variability by using block (complete or incomplete) designs. However, a large amount of spatial variability still remains unaccounted for, and this may lead to erroneous conclusions. To further capture this unaccounted-for variation (which is mainly due to intra-block variation), yield data from variety yield trials can be analysed using various spatial models. Singh *et al*. (2003) showed that spatial models add considerable value to trials; the 'best' spatial models gave efficiency values of over 330 percent in winter-sown chickpea, 140 percent in lentil and 150 percent in barley trials. Furthermore, the use of these best models resulted in a change in the ranking of genotypes (on the basis of mean yield), which therefore resulted in a different set of genotypes being selected for high yield. It is recommended that

(1) incomplete block designs be used in variety trials; (2) the Akaike Information Criterion (developed by Akaike under the name of "An Information Criterion" (AIC) in 1971 and proposed in Akaike (1974) as a measure of the goodness of fit of an estimated statistical model) be used to select the best spatial model; and (3) genotypes be selected after the use of this model. The selected model would most effectively account for spatial variability in the field trials, improve selection of the most desirable genotypes, and therefore improve the efficiency of breeding programmes.

Additional information on this issue, also with regard to useful software, is given in Chapter 20.

3.6 MULTI-ENVIRONMENT TRIALS

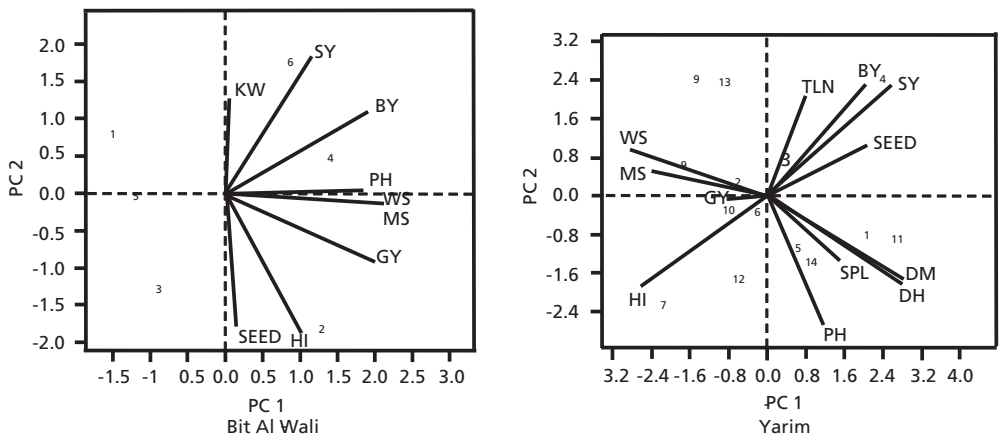
The last stage of a plant breeding programme aims also at understanding the reaction of the breeding material to a multitude of environments, i.e. locations, years, and possibly different types of agronomic management. During this process, the amount of breeding material is progressively reduced, and the number of locations and the number of replications per location is progressively increased. In the first level of yield testing the general tendency is to have as many locations as possible (the main limiting factor being the amount of seed available), sacrificing number of replications per location. Because of the limited precision of these trials, most breeders will mostly do negative selection, i.e. discarding the obviously inferior breeding material. As the material is advanced, the precision of the trials also increases. At the end of the testing phase, and for the surviving breeding material, data are available from a number of locations and years, which can be analysed with one of the techniques described in Chapter 20.

When METs are conducted in farmers fields, which is common in some conventional breeding programmes (in Australia, for example), the breeder may face organizational issues different from those in a research station (Chapter 9). METs planted in farmers fields are not yet participatory plant breeding because, as is the case for the Australian breeding programmes, farmers only make land available against the payment of a rent and do not participate in decisions related to the selection of breeding material.

One important aspect of a MET is the ability to subdivide Genotype × Environment interaction into Genotype × Location (G×L) and Genotype × Year (G×Y) interactions. Distinguishing these interactions is important because the two differ in importance with regard to plant breeding. While G×Y interactions are largely unpredictable, G×L interactions can be predicted. In the case of G×L interaction, therefore, it is essential to assess both its magnitude (relative to G) and its repeatability over time. Such an assessment allows the target population of environments to be divided into subsets in a way that ensures that there is a high degree of repeatability or consistency (Kempthorne, 1952) of G×L interaction between subsets and a low degree of repeatability within subsets. Eventually this leads to the identification of high-yielding, stable genotypes adapted either specifically or widely, and to clusters of response-similar environments, which can contribute to defining a selection strategy and to locating a small number of optimal selection sites for future breeding. These topics are extensively discussed in Chapter 20.

The use of biplots as means to graphically display genotype adaptation patterns and environment similarity for GE interaction effects is discussed in Chapter 20.

FIGURE 3.1
Biplots of farmers' preferences in barley grown in two villages in Yemen (6 and 14 varieties, respectively)



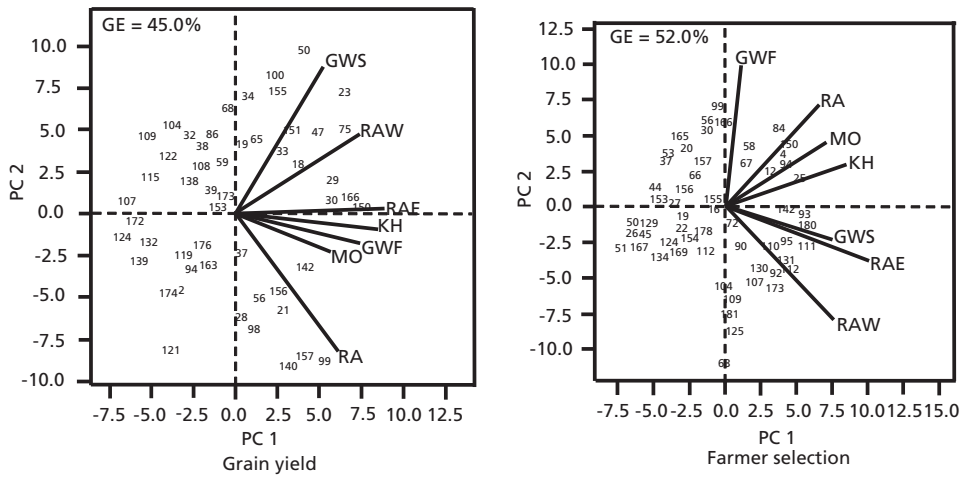
Key: PH = plant height; KW = 1000-kernel weight; SEED = number of kernels/spike; BY, GY and SY = biological, grain and straw yields, respectively; HI = harvest index; DH = days to heading; DM = days to maturity; TLN = tiller number; MS = male selection; WS = female selection.

Biplots, such as those envisaged by Yan and Tinker (2005), can also be useful in PPB programmes to assess (1) the traits used by farmers as selection criteria; (2) the consistency of selection criteria across environments; and (3) whether locations that show repeatable G × Y interactions and that therefore could be lumped together as one subregion (or mega-environment), actually differ in farmer preferences.

An example of using the biplot to assess which traits are actually used by farmers as selection criteria and the consistency of selection across environments in given in Figure 3.1. A narrow angle between the vectors for farmer selection (MS for the males and WS for females) and the vector for a plant character indicates a strong preference for that given character. This is the case of plant height in Bit Al Wali, where both men and women selected for tall plants, and to a slightly less extent for grain yield. It can be noted that in the case of the second village, the preferences were reversed, the wide angle between the vectors for farmer selection and those for plant height suggesting that farmers in Yarim selected for short plants and for early heading and maturity, as indicated by the direction of the vectors of these two characters opposite to the vectors for farmers' selection.

Figure 3.2 shows an example of differences in locations clustering depending on whether grain yield or farmers' preferences are used. On the basis of grain yield, four locations, namely MO, GWF, KH and RAE, are closely correlated; they are likely to represent a similar environment as they discriminate similarly among genotypes. If this is repeatable over time, then it can be argued that any one of the four will be sufficient to represent that given macro-environment, thus leading to a considerable saving in resources. However, only 2 of the 4 locations (MO and KH) are closely correlated also for farmer preference, while GWF and RAE are independent from each other and weakly correlated with the pre-

FIGURE 3.2
Biplots of grain yield and farmers' preferences for 182 barley genotypes grown in six farmers' fields and one research station in Jordan (not all genotypes are shown)



GWF = Gweer farmer field; GWS = Gweer research station; RA = Rabba; KH = Khanasri; MO = Mohay; RAE = Ramtha East; and RAW = Ramtha West.

vious two. Therefore, if these patterns are repeatable over time, it would be advisable to only drop either MO or KH.

3.7 CONCLUSION

In a typical breeding programme it is possible to recognize three distinct stages: the creation of genetic variability; selection of the desirable gene combinations; and the final testing of these desirable gene combinations.

In a truly participatory breeding programme, farmers participate in all the three stages. When farmers participate in only the last stage (as often is the case), it is more appropriate to talk of participatory variety selection (PVS). There are important conceptual differences between the two. PVS is selection among (usually only a few) finished or nearly finished varieties, such as when farmers choose from on-farm variety trials, which are the very last stage of a breeding programme, and, very importantly, it is a linear process. In contrast, in PPB farmers participate in selection when genetic variability is at or near its maximum, such as selection between or within early segregating populations. Also, contrary to PVS, PPB is a cyclic process.

Ultimately, a participatory plant breeding programme can use and benefit from the use of the most advanced experimental designs and analytical tools.

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CHAPTER 4

Methodologies for priority setting

Eva Weltzien and Anja Christinck

4.1 WHY DO WE NEED METHODOLOGIES FOR PRIORITY SETTING IN A PLANT BREEDING PROGRAMME?

Productivity improvements have been the key objective of most plant breeding programmes to date: international, national, private and public alike. Other major breeding objectives are indirectly related to this goal: resistance to pests and diseases, for instance, or adaptation to abiotic stresses (such as drought or low soil fertility), and aim at increasing or stabilizing yield or to allow higher production under certain environmental conditions.

Another group of 'classical' breeding objectives focus on adding 'value' to crops by improving their qualities for industrial processing, their storability, or by meeting certain consumer preferences. Some breeding programmes concentrate on increasing the nutritional value of staple food crops, an approach that is also known as biofortification (HarvestPlus, 2007).

Increasing the yield of important food crops was seen as the answer for overcoming food shortages and reducing hunger in the world. In fact, the production volume per hectare of some major food crops has increased about threefold in the last 70 years, partly as a result of plant breeding and partly due to intensification of farm management (Becker, 1993). In recent years, however, evidence has been mounting that the global availability of staple food alone is not sufficient for reducing hunger and malnutrition. Food insecurity is closely related to poverty in general: even if food is available, many poor people, including poor farmers, lack access to it. The alleviation of poverty has therefore become a key development goal. It is at the top of the agenda for many development organizations, both governmental and non-governmental, and also for international agricultural research centres. In view of this goal, international breeding programmes and their national partners have been compelled to redefine their programme objectives and specific targets. Crop breeding programmes, for instance, must be re-oriented towards the needs of poor farmers and other specific user groups. However, user differentiation and gender considerations are new concepts for many breeding programmes; developing new and 'better' varieties was assumed to be a largely user-neutral technology.

Furthermore, the benefits from newly developed varieties are not evenly distributed; in some regions, for example sub-Saharan Africa, where poor soil fertility and erratic rainfalls limit the potential for agricultural production, there has practically been no yield increase in major food crops in the last 20–30 years (FAOSTAT data, 2006). In such regions, farmers have often developed complex farming systems and strategies for reducing environmental risks. However, social, political and economic change can weaken such systems, leading to instability and overexploitation of the natural resources. Plant breeding for such situations requires different approaches: approaches that are based on a deep understanding of the functions of crops within the entire system, including farming, nutrition, local knowledge and technologies. Setting priorities for such programmes needs to be forward looking, as it may take at least ten generations before new products become available. They then need to be adapted to farmers' needs and production systems. Simple strategies, such as improving yield by increasing the ratio of the edible part at the expense of other plant organs (i.e. foliage, roots), do not generally work under such conditions. For example, certain 'minor' characteristics may

BOX 4.1

The value of pearl millet straw in drought years

In western Rajasthan, drought occurs so regularly that farmers have developed their strategies to cope with it. Many farmers, even though interested in new varieties for testing and experimentation, grow traditional pearl millet landraces. In good years, the yield of the landraces is moderate, but their real value is revealed in severe drought years: even if the grain yield may be strongly reduced, they produce some grain as food and biomass for feeding the animals. Many modern varieties fail totally under such conditions, producing neither straw nor grain.

There are several possibilities for people to find grain for human nutrition: some may have stored a surplus from previous years, or one can do labour work and buy grain from other regions in the market. In severe situations, food aid may be distributed by governmental or private aid agencies. But starvation of animals hits a farmer family hard for years; the animals are an important source of income, besides providing dung, draught power, milk or wool for the family and the farm.

be re-lated to environmental adaptation, or non-edible plant parts may have a high value in particular situations (see Box 4.1).

Another point receiving increasing attention is the conservation of agricultural biodiversity. Many countries have signed the Convention on Biological Diversity (CBD), or the legally binding International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). It is now widely recognized that industrialized farming has led to significant losses of biodiversity in agricultural systems. This is due to the use of only a few, widely adapted, varieties; the narrow genetic base of the breeding materials; and testing and release procedures allowing only the dissemination of a limited number of relatively uniform varieties. Economic considerations are one reason why previously selected breeding material is used as much as possible, but it is thus reducing the genetic diversity among the newly developed varieties. Landraces and wild plants are not incorporated as much as they could be, because it may take more time to derive stable and uniform varieties from such material, thus increasing the cost of such programmes (Haussmann *et al*., 2004). Furthermore, the business economics of breeding firms require a large geographical distribution of varieties within a few years, which conflicts with biodiversity considerations and other aspects of regional differentiation, such as respecting food culture and consumer preferences. Locally important crops often do not reach the scale of distribution that is needed by breeding institutions for them to invest in new varieties. However, as a result of international commitments, national and international breeding programmes are obliged to initiate efforts for broadening the genetic base of breeding materials, according to the Global Plan of Action (FAO, 1996), and the International Treaty for PGRFA (ITPGRFA, no date).

Decentralized breeding programmes, based on local crop germplasm and seed distribution systems, in contrast, could be an important step towards increasing the level of biodiversity in farmers' fields. Moreover, the goal of conserving agro-biodiversity could effectively be linked to efforts to increase food security and reduce poverty: Many 'minor' or traditional crops

(or crop varieties) have outstanding nutritional qualities, are well adapted to marginal conditions and low input farming, or open up possibilities for income generation (IPGRI/GFU/MSSRF, 2005).

Thus, breeding programmes today often have to be designed in a manner different from the past. To meet the above-mentioned new goals, they tend to be less centralized, more targeted towards specific user groups and often use different germplasm. However, this is not all. To obtain impacts beyond a very local scale, approaches have to be developed that address large geographical areas while at the same time respecting agro-ecological and socio-cultural differences. This usually requires cooperation among different organizations that work at different scales, and often have diverse agendas and backgrounds. Consequently, methodologies for priority setting have to be adapted for such cooperation to make the process transparent and acceptable for all stakeholders.

The management of social cooperation, learning and decision-making processes is, as such, new for most plant breeders and their institutions. However, experiences exist from other disciplines, particularly social and economic sciences; here one can build on fundamental expertise in the fields of knowledge systems, communication, social learning and management (Leeuwis, 2004; Manktelow, 2003).

4.2 PARTICIPATORY PLANT BREEDING

The concept of participatory plant breeding (PPB) emerged in the late 1980s as part of a general development in participatory research methodologies during that period. Increased user orientation and more efficient allocation of research funds; higher adoption rates; a close relation to local cultures, knowledge and skills; empowerment of farmers; and overcoming typical limitations of 'science' in the development context—all these factors are the potential advantages of participatory plant breeding (Ashby and Sperling, 1995; Weltzien *et al.*, 2003).

PPB includes all approaches to genetic plant improvement involving close farmer–researcher collaboration. The term particularly refers to active involvement of farmers in at least one of the stages of a plant breeding programme, including setting objectives, generating variability, selecting and testing, as well as seed production and distribution.

This active involvement of farmers can take different forms. Farmer participation can be consultative, if farmers are interviewed on agro-ecological issues, or on the performance of test varieties. More active forms of farmer participation include, for example, trial management, selection, priority setting and the development of action plans, or the overall management and implementation of the project (Farnworth and Jiggins, 2003; Lilja and Ashby, 1999). Which degree of farmer participation is appropriate and in which phase of a breeding programme depends largely on the goals of the programme, as well as the type of improvements needed, and it is thus also an issue for priority setting (see Section 4.5 below, under Roles and Responsibilities).

4.3 PRIORITY SETTING AS AN ITERATIVE PROCESS

Setting priorities is an important part of professional plant breeding work. Time and resources are usually limited, and they have to be allocated in a rational way in order to reach the goals of the breeding programme. Thus, considering issues and methodologies for priority setting is a necessary step for any plant breeding programme, irrespec-

tive of the degree of farmer participation or the institutional setting. However, little has been reported to date on methodologies for priority setting in plant breeding programmes. Resource allocation, primarily during the phase of testing experimental cultivars, has been researched intensely, usually based on models for maximizing genetic gain, thus focusing on one or two key traits (e.g. Cooper and Byth, 1996).

We regard priority setting as an iterative and progressive process that will be considered at many stages during a plant breeding programme, not only in the project planning phase. It is often not possible to anticipate all the options that may emerge in the course of the research process. Priority setting methodologies should therefore become part of the regular project work, in a way that allows adjustments and further development of goals and priorities as the project work evolves.

In the following sections we will look at issues for priority setting first, and then suggest methods and communication tools that could help to achieve a transparent process and productive outcomes.

4.4 ISSUES FOR PRIORITY SETTING

Clear priorities need to be set for a number of issues. The **goals** are the guiding principles for priority setting in any project of a defined duration, scale and scope. At the same time, the goals themselves are also an issue for priority setting, as complex, conflicting or too general goals are not likely to be reached through technical plant breeding work alone.

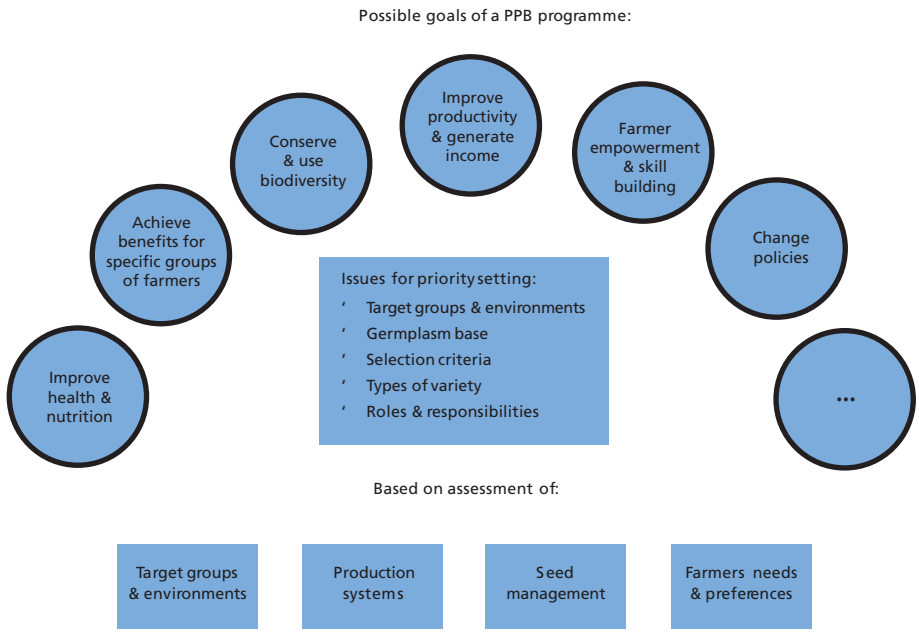
For plant breeding programmes, it is vital to define the **target group(s)** and **the target environment(s),** i.e. production conditions under which the newly identified varieties should perform better than existing cultivars, and the specific needs of the target group of farmers. Closely linked to this are priority traits to be used as **selection criteria.** To achieve good progress from selection, the **germplasm base** must be chosen appropriately, based on profound knowledge of the available options. It is also important to discuss what **type of variety** might be the most appropriate for achieving the project or programme goals. Part of this issue is also to address the question of intravarietal diversity: how much of it would be beneficial or necessary, and for which traits. An issue that is often left until activities are planned is the identification of key **roles and responsibilities of partners**. However, since different options for sharing responsibilities between partners have a major impact on some of the goals, it is important to consider them from the outset of the breeding programme. The following sections explain in more detail how the different issues for priority setting for a breeding programme relate to the overarching goals in specific situations.

4.4.1 Goals as a basis for priority setting

All breeding programmes have at least one goal related to improving production, such as yield, yield stability or a higher product value. Many PPBs have additional goals, such as the conservation of local diversity, skill building and empowerment of farmers, policy and regulatory changes, increasing research efficiency, or benefits to specific users. Many of these goals tend to be implicit and depend on the institutional background and on the 'history' of the breeding project.

Each organization and institution has their own implicit goals that are not always easily communicated. Thus close interaction, exchange visits, and joint planning workshops that are held variously in the

FIGURE 4.1
Issues for priority setting in a plant breeding programme in relation to the overall goals and based on the assessment of target groups and environments, production systems, seed management, and farmers needs and preferences



Source: Weltzien, 2005.

different partners' workplace (e.g. research station, village, trading place) are important to achieve a mutual understanding of the different partners' perspectives. It is also understood that the relative importance of the different goals may change as the project and, foremost, the partnership advances and evolves.

If the project work involves close interaction between farmers or farmer organizations and researchers, it is particularly important to clarify the goals from the project planning phase. For many farmers, it is not easy to understand what scientists do and how research is organized. As a consequence, they may be tempted to overestimate the direct effects of the research on yield or income generation, or they may even expect other benefits from the cooperation, which cannot be fulfilled by a breeding programme. Such general aims of the people could perhaps better be addressed by activities other than plant breeding, or by establishing partnerships with marketing organizations or food processing companies (and including their specific goals into the breeding programme).

From goals to priority setting

The goals have been described as the guiding principles for priority setting. At the same time, the priority setting process builds on understanding the present situation, anticipated changes, and farmers' needs. A detailed analysis of the production environment is required, including existing

varieties and how they are used by farmers, their preferences and relevant resources (i.e. local knowledge, skills, germplasm). In particular, it is necessary to identify the major constraints to production increases and income generation. Participatory methods for such situation analysis have been described in detail by Christinck, Weltzien and Hoffmann (2005). An open dialogue in the course of which all partners evaluate potential options and obstacles for future breeding activities could then follow (see Section 4.6 of this chapter). This approach is graphically summarized in Figure 4.1.

4.4.2 Target groups and target environments

Identifying the target environment and target group in view of the overall project goals is generally among the first strategic decisions to be taken in a plant breeding project. We therefore suggest a few subjects for consideration, which refer to agro-ecological as well as socio-economic factors.

Broad versus narrow adaptation, and the impact of PPB

The issue that certain plant types or varieties may perform differently in different environments is called 'genotype by environment interaction' by plant breeders. In general, most plant breeders tend to give preference to those populations that perform well under a wide range of conditions; this ability of plant populations is known as 'broad adaptation'.

Broadly adapted varieties are also the prime matter of interest for seed companies, as the potential profit from the entire release and multiplication 'business' is usually related to the scale of distribution. However, these varieties, if tested on research stations in multi-locational trials, may fail under the conditions of poor farmers working with limited resources and under marginal agro-climatic conditions. Ceccarelli, Grando and Booth (1996) and Ceccarelli *et al.* (2000) have shown theoretically and practically that interactions between genotype and environment can be positively exploited if the selection is done in the target environment, e.g. farmers' fields. Farmers as well as scientists successfully selected populations or experimental lines that produced better under the farmers' conditions than other varieties grown previously by those farmers. Experiences of other research groups, with various crops in differing natural and socio-cultural environments, support this understanding (Goyal, Joshi and Witcombe, 2001; Mekbib, 1997; Sperling, Loevinsohn and Ntabomvura, 1993; Weltzien *et al.*, 2003). Narrow adaptation to specific conditions, leading to the selection of many different cultivars for various conditions and purposes, is often regarded as an advantage of the PPB approach: it serves specific needs of farmers and enhances the level of agrobiodiversity in farmers' fields (Sperling, Loevinsohn and Ntabomvura, 1993; Joshi and Witcombe, 2001).

However, a possible criticism regarding decentralized plant breeding programmes could be that, due to the focus on specific, often marginal environments, and only the local importance of the varieties developed, their impact remains insignificant. Only a very few farmers who produce mainly for their own subsistence and modest requirements would profit from the activities, and this would never justify the breeding efforts, let alone the cost of official variety release and seed multiplication.

At the same time, there are also cases where varieties developed through PPB programmes are not necessarily so narrowly adapted. In Nepal, for example,

a rice variety selected by farmers in a high-altitude environment was adopted by many farmers in the mid-altitude regions as well (Joshi, Sthapit and Witcombe, 2001). Also, rice varieties developed in a participatory breeding programme in Nepal were superior to check varieties in a region of Bangladesh, where rainfed agriculture prevails (Witcombe *et al*., 2005). Obviously, much depends on the characteristics of the varieties, the conditions under which they were selected and the limitations that were addressed and overcome through the plant breeding activities. Thus, information on target regions and how representative these are for other farmers of a larger area will be of vast importance for the later impact of the project.

Identifying, specifying and delineating the target environments for a breeding programme more precisely is often done by analysing multi-location trials through which a broad range of potential varieties for a region can be evaluated. Calculating correlations between performance traits from the different testing sites usually gives an initial impression about the differences between the sites with respect to adaptation (Atlin, Paris and Courtois, 2002). If sufficient data is available, or can be generated during the course of the project, more complex statistical tools can be employed by breeders in order to delineate target environments and develop a selection and testing strategy for new varieties (e.g. Cooper *et al*., 1999; Cooper and Byth, 1996; Annicchiarico, Chapter 20). These statistical tools do not require farmer participation, but give a much more realistic assessment of the situation if the trials used for these analyses were conducted by farmers in farmers' fields, using farmers' selection and evaluation criteria. Similarly, farmers' description of requirements for adaptation to a specific zone can be a useful input, which could actually save efforts on long-term expensive experimentation and analysis (van Oosterom, Whitaker and Weltzien, 1996; van Oosterom *et al*., 2006).

New crop varieties: for people or for environments?

In general, plant breeders tend to focus their breeding strategies on regions and agro-ecological conditions: so-called 'target environments'. The idea that people belonging to different social groups (even when working under similar agro-ecological conditions) may have different requirements for seed and varieties, so that we have to target our work not only to natural, but also to social and economic conditions, may be less apparent. In this section, we therefore enter into more detail and describe why we need to explore and integrate both aspects: defining a target environment not only from natural but also from socio-economic perspectives.

General agro-ecological conditions can be described with relatively few parameters, which are usually available from secondary sources, such as general physical maps, soil maps and meteorological data. With this information, we can distinguish agro-ecological zones according to:

- different altitudes;
- different soil types;
- different rainfall patterns;
- availability of irrigation water;
- etc.

Depending on the scale for which this type of information is available, this analysis will result in relatively large zones that appear more or less homogenous. However, this is seldom true in the farmers' reality. Even farmers in relatively favourable agro-ecological regions or irrigated areas often have land that is of poor quality, due to local

constraints such as stones, rocks, gravel or hard subsoil layers, hilly land, or poor quality or limited availability of irrigation water. Therefore, marginal agro-ecological conditions can be found surrounded by more favourable environments, and depending on a farm household's total land area and the location of the fields, these conditions can be of considerable importance (see Box 4.2). The farmers' requirements for seed and varieties depend directly on the conditions present on their land, and on the limitations and constraints they have to face in their daily work. Thus, it is indispensable to complement agro-ecological information from secondary sources with local information, including soil types, irrigation water and typical constraints to agricultural production. Care should be taken to include information from various social groups, as land quality and access to natural resources often vary for different people in a village.

Furthermore, the same natural and agroclimatic conditions can pose different problems and opportunities for people, depending on other resources they possess. For example, soil constraints may have different importance depending on the machinery used by a farmer, and the availability of groundwater for irrigation purposes helps only if a farmer family can afford the irrigation equipment and operation costs. Expensive seed and other costly inputs may not be accessible for poor farmers, so that they have a preference for varieties that can be multiplied on farm and successfully grown under low-input conditions, even in a favourable agro-climatic environment. These examples show how economic factors influence the farmers' needs and preferences regarding crop varieties.

Social factors may be of equal importance. People belonging to different social groups may have different needs, preferences and access to resources. In many cultures, for example, women and men have different responsibilities with regard to farming, nutrition and income generating activities, which may result in different preferences. Ethnic groups, clans or castes may be specialized in certain agricultural activities, such as pastoralism, general farming, horticulture or cultivation of trees, and

BOX 4.2

Soil quality and settlement patterns

In some parts of the world, we can observe some level of coincidence between agro-ecological conditions and settlement patterns, so that distinct social groups live and work under different agro-ecological conditions even in the same village. Examples are:

- Remains of feudal systems: The kings and members of the nobility usually possessed the best lands and the rights to access water and other natural resources. The 'ordinary people' worked on marginal lands.
- Remains of colonization: In the process of colonization, indigenous people were forced to leave their land and settle in less favourable conditions.
- Migration due to wars or disasters: Refugees and other 'newcomers' are often allocated marginal lands that are not used by the original population.

These settlement and land use patterns can persist for generations

Source: Christinck and Weltzien, 2005.

cooperate according to traditional rules and rights.

One practical option to clarify and limit the target environments for a plant breeding programme is to identify with farmers the variety that the new programme needs to replace in order to be successful. In cases where this is possible, the range of distribution of this variety may then be considered the target environment(s) for the new breeding programme. In areas of high varietal diversity, this may not be so evident, and may require more understanding about which varieties or group(s) of varieties play what role in the production system and livelihood of the target group of farmers. In other situations, it may actually be most useful to add a new variety to the spectrum of varieties already grown by farmers, with specific new uses or adaptation characteristics, such as sorghum with good malting qualities to meet the needs of an emerging industry.

In summary, farmers may have different needs and preferences regarding crop varieties and specific traits in relation to their economic situation and their social group(s). Therefore it will be important to develop an understanding of how natural as well as socio-economic factors relate to the farming practices of different farmer groups, particularly in view of their use of varieties and needs for specific traits. The decision about the target group of farmers determines largely which project goals can be achieved, which is decisive for the 'success' of a project. Since the decision on target groups guides many subsequent steps in the priority setting process, it should be a primary concern for plant breeders. Similarly, evidence from impact assessment studies has shown that adoption of new varieties is often limited because the target group and their specific needs and preferences were not adequately considered by breeding programmes (Weltzien *et al*., 2003; Witcombe *et al*., 2005).

4.4.3 Selection criteria

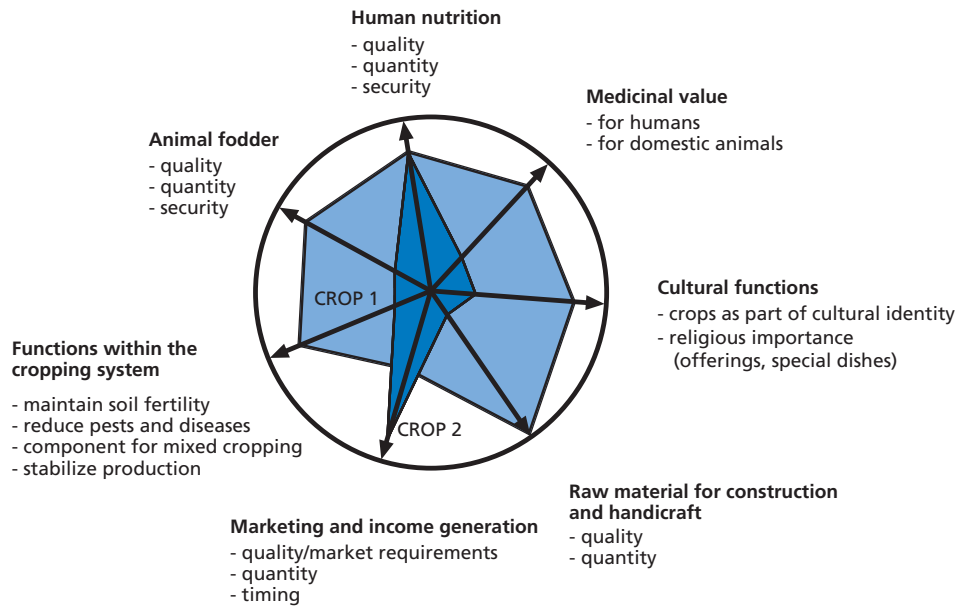
Once the project goals as well as target group and environments are identified, decisions about the type of improvements needed and the selection criteria will come into play. Looking towards future options requires a sound understanding of the situation and the conditions under which newly developed varieties will need to function. This will be the basis for developing new, creative options.

Functions of crop varieties in the farming system and related selection criteria

Crop varieties, particularly those with a long history of cultivation in a given region, are not only adapted to natural conditions, but also to the needs of the people and their cultural preferences. They can fulfil a wide range of functions within the entire system of farming, nutrition and cultural life of a farmer family, and provide important by-products (see Figure 4.2). However, as many rural areas are in a process of rapid socio-economic change, improvements in specific traits can be interesting for the farmers. In most cases, this will depend on the economic importance of this particular trait, and the overall acceptability of the variety with regard to other important traits. This figure can also help us to think about the type of improvements needed to achieve the project goals.

As a first step, we should gain some knowledge on the farmers' variety portfolio, their use of varieties and the strengths and weaknesses of these varieties in relation to functions and project goals. This characterization of varieties should be based on farmers' knowledge and perceptions.

FIGURE 4.2
Functions of crops within a farming system



Crop 1 is a typical multipurpose crop with high importance for most functions (except marketing), whereas crop 2 is a food crop important for nutrition and marketing, but not for other functions mentioned
Source: Christinck, Dhamotharan and Weltzien, 2005.

Various tools for entering into dialogue with farmers on variety characterization and use have been proposed by Christinck, Weltzien and Dhamotharan (2005). Furthermore, understanding farmers' own seed selection and the underlying criteria will give us important keys for the types of improvements farmers are looking for.

Some selection criteria are largely determined by the requirements of adaptation to the target environment, e.g. flowering date, resistances to specific pests and diseases, or to abiotic stresses such as soil acidity. Other selection criteria are determined by the technologies farmers are using, such as ease of harvesting, transportability, manual threshing, or by the requirements of the farming system, e.g. mixed cropping and fodder use. Furthermore, selection criteria may also be related to culinary preferences, such as taste, usefulness for certain preferred dishes, to useful by-products (i.e. construction material) or to market requirements, e.g. grain colour and shape. In most cases these criteria must meet a certain threshold level of acceptability.

Experience from PPB projects has shown that farmers often select for many criteria simultaneously, and in this way can indirectly achieve considerable yield increase. This seems to be mainly related to the farmers' ability to anticipate the performance of certain plant types under specific conditions that are well known to them (Sperling, Loevinsohn and Ntabomvura, 1993; Christinck, vom Brocke and Weltzien, 2000).

However, for professional plant breeders, a detailed evaluation of each and every trait

that might be important for farmers will lead to a dead end. Resources for testing and evaluating new germplasm or breeding material are always limited. The more criteria that are included in a selection programme, the less effort can be spent on each of them, and thus less progress tends to be obtained from selection. Thus, a guiding principle in the choice of selection criteria should be to keep them to the minimum necessary. The more focused and clear the targets for selection, the greater are the chances of achieving them. We find here an excellent option for cooperation between farmers and scientists in a breeding programme. Farmers can more efficiently select those materials that are overall compatible with their situation, farming system and marketing requirements and preferences, whereas scientists can be most effective in assembling appropriate germplasm with the traits desired by the farmer and in selection for a limited number of critical traits.

Heritability of traits and environmental adaptation

Formally trained plant breeders tend to classify traits by the complexity of their genetic control. They differentiate highly heritable traits with simple genetic control from genetically complex traits with low heritability, along a continuum of increasing complexity, and thus decreasing genetic control or heritability (see Chapter 2).

Highly heritable traits with simple genetic control tend to be mostly descriptive traits, such as colours of the grain or other plant parts, hairiness, key aspects of crop duration or flowering date, plant height and some types of disease resistance. While some of these traits are key factors for the adaptation of a variety, such as flowering date or disease resistance, many others are more related to what is intuitively often thought of as a preference: something visual, qualitative and not really associated with productivity or adaptation. Most of these traits could actually be incorporated into existing varieties by backcrossing, if a source for the desired trait, i.e. a gene, exists in the breeders' collection.

Complex traits have a low heritability because their expression is highly influenced by environmental factors, i.e. the conditions in which the variety is grown. Many of these traits also tend to show sizeable amounts of genotype × environment interactions, i.e. the expression of a trait in specific varieties depends on the conditions in which the trait is being evaluated (see Chapter 20). One example would be a variety which responds well to fertilizer; its yield under high fertility conditions could be higher than that of a local variety, whereas the local variety would outperform this variety under low soil fertility conditions. This example shows clearly that identifying yielding ability as a key preferred trait is of little relevance. However, what is important is the specification for which kind of growing conditions a higher yield performance is being sought by farmers. This type of specification is necessary for most of the complex, productivity-related, traits, as their assessment cannot be dissociated from the conditions under which they are evaluated.

Another example of a selection criterion, which is often high on farmers' lists of preferences, but usually very difficult to assess, is drought tolerance. The first problem is that a trait like drought tolerance may mean very different things to farmers, to crop physiologists or to breeders, and would thus entail very different ways of assessing it, from physiological measurements of drought response at the biochemical, plant tissue, plant organ or

whole plant level, through to productivity under specific drought conditions. Practical breeding experience with drought tolerance has shown that it is of key importance to ensure that the crop's water requirements match the periods of water availability in the target production system. It is thus important that the nature of such complex traits of adaptation are well understood before deciding to use them as a focus for selection and variety improvement. Traits that cannot be assessed or evaluated with the necessary precision in the planned project should thus not be included as selection criteria. Before it can become a selection criterion, some research might be necessary to find appropriate ways of assessing or measuring such a trait.

New selection criteria can lead to new options

It could be a 'breakthrough' for farmers if some well known traits of already existing varieties could be improved. However, in some situations, radically new options can emerge if totally new selection criteria are taken into consideration. For example, in regions where crop production has so far been merely subsistence oriented, traits important for food processing industries could lead to new marketing options. Totally different plant types with different growing behaviour, such as extra-short growing cycle or extra-tall plants, could help farmers to diversify their farming systems.

Such extreme changes can often not be envisaged by farmers, if they have no practical experience with such varieties. Thus, it is an important task for plant breeders to find out (together with farmers or based on a thorough understanding of the farming systems) which new options could really be beneficial and interesting for the farmers.

On-farm or on-station evaluations of exotic varieties, excursions to food processing plants and visits to other regions could be a way to start developing radically new options with farmers.

Success from selection

A clear target is essential for the effectiveness of any plant breeding effort. The clearer and the simpler the target, the greater are the chances of achieving it. If the target, and thus the priorities for selection, can be simplified, then the full selection effort can be focused on those key traits. Such targeted selection efforts have a much higher rate of success and of progress from selection than programmes that have to consider multiple and very complex traits as selection criteria. Therefore, investing some time at the beginning into the development of clear priorities for selection can help enormously to increase the overall efficiency of a breeding programme. This is why most PPB programmes put great emphasis on understanding farmers' preferences and needs (Weltzien *et al*., 2003).

Selection priorities may be different for different groups of farmers. Transparency here can help to compare the identified selection priorities once again with the overall project goals, and then decide how (and with which group of farmers) to best achieve them. Tools for discussing different options and trade-offs with farmers will be presented in the last section of this chapter.

4.4.4 Choice of base germplasm

Selection can only be successful if there is sufficient diversity from which to choose. It is thus clear that the selection criteria and the choice of germplasm are intimately linked. Traits for which no genetic variability is available cannot be considered for genetic improvement. Similarly, the extent

of diversity available for selection largely determines the success of the selection programme. This is particularly important in view of the first guiding principle for prioritizing selection criteria, namely to keep criteria to the minimum necessary.

Using local germplasm as breeding parent: a way to increase the acceptability and adaptation of new varieties

One basic approach for keeping the number of selection criteria to a minimum is to identify base germplasm that already has most of the traits expressed at the threshold level or above, but is variable for the major trait targeted for improvement. Many PPB programmes have been very successful in this respect, because they did use the local germplasm and farmers' knowledge of it for this purpose. By using local germplasm, most of the traits for adaptation and use are already expressed at this threshold level, and the novel germplasm can be chosen to introduce new variability specifically for improving one or two key traits, e.g. reducing the period from planting to flowering, or increasing yielding ability, or stover quality, or resistance to a major pest or disease.

Plant breeding and biodiversity conservation

The choice of germplasm is also a key issue for achieving goals related to biodiversity conservation. If used successfully in plant breeding programmes, there are much better chances of 'endangered' germplasm being preserved, compared with other approaches focusing on conservation *per se*. If diversity conservation is a primary goal of a plant breeding programme, a very good understanding of the nature and functions of this diversity for the target group needs to be achieved. Assessing local diversity in a participatory research process can, as such, contribute to raising awareness about the usefulness of this diversity among participating farmers and scientists, and thus increase the chances for future use of this germplasm. However, the goal of increasing biodiversity in farmers' fields does not necessarily require a focus on local and traditional germplasm. Particularly in those regions where a major part of the local diversity is already lost, a plant breeding programme could also be based on material from elsewhere, showing enough diversity in traits that have been identified as useful for the target group of farmers.

Any adoption of new varieties by farmers will change the portfolio of varieties available in a village community. This may provide interesting new options for some farmers, and possibly disadvantages for others. Such developments can often not be anticipated fully. Unintended (negative) outcomes for some farmers can be reduced by ensuring the multiplication and access to seed of the original varieties, for example through strengthening seed exchange networks, institutionalizing seed fairs or community seed banks.

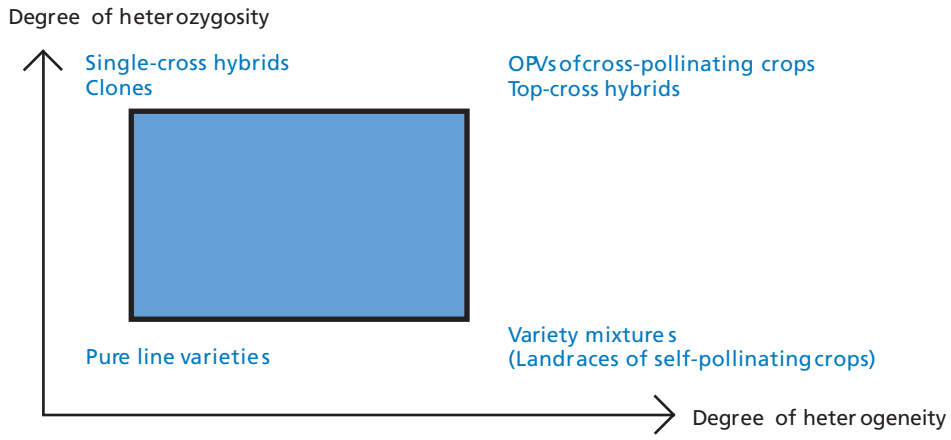
4.4.5 Types of variety

What type of variety will be developed in the course of a plant breeding programme has important implications with regard to the biodiversity in farmers' fields and to the options farmers have to use seed of this variety for re-sowing, selling, exchange and their own breeding activities. These aspects touch the overarching goals of the breeding programme, and are thus important for consideration in the process of priority setting.

Variety types and agrobiodiversity

Varieties can have very different genetic structures; they can differ in the degree

FIGURE 4.3
Degree of heterogeneity and heterozygosity in different types of varieties



Source: Weltzien and Christinck, 2005.

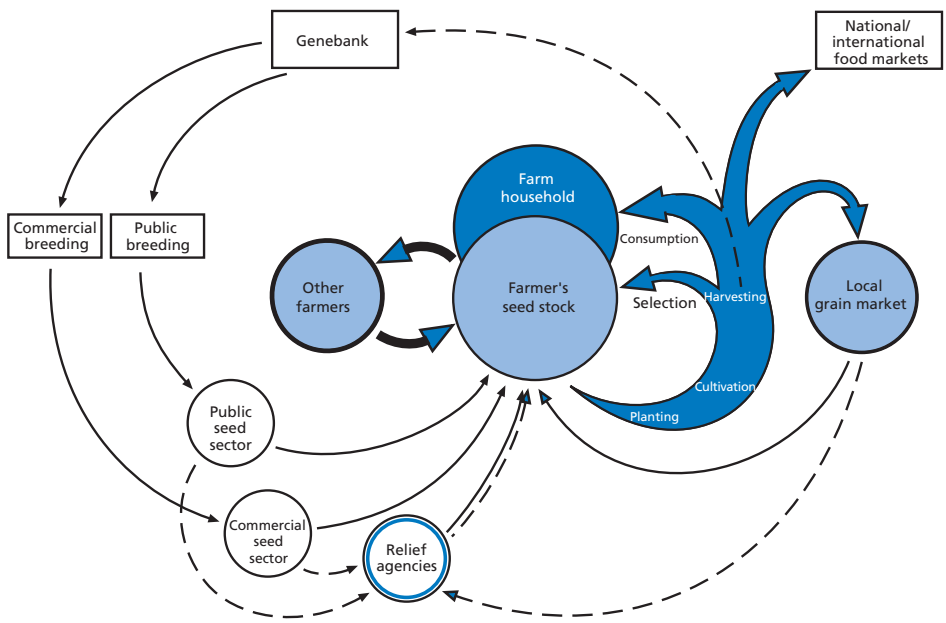
of variability maintained within the variety and in the degree of heterozygosity, with important implications for the ease of reproduction (Figure 4.3).

Pure line varieties of self-pollinated crops are homogenous and homozygous, and could theoretically just be made up of one single genotype that can easily be reproduced. Single-cross hybrids may also be made up of only one genotype (the offspring from a cross between two homozygous parental lines). However, they have very high degrees of heterozygosity and cannot easily be reproduced by farmers. Other types of hybrids will have different levels of diversity within them, such as top-cross hybrids, where one parent is an open-pollinated variety of a cross-pollinated crop. Open-pollinated varieties have a high degree of intravarietal diversity. Heterozygosity is also present in such varieties, depending on the out-crossing rate of the crop and the diversity of alleles for genes in the population. Open-pollinated varieties can be reproduced easily if contamination with pollen from other varieties can be prevented. Variety mixtures (multi-line varieties) or some landraces of self-pollinating crops may be both homozygous and heterogeneous. They are reproducible if natural selection pressures do not differ very much from the conditions under which they were developed, so that specific types or components will not disappear.

Furthermore, a breeding programme could also reach diversity-related goals through developing a number of varieties for specific conditions and uses, and for various user groups. This approach has important implications in the longer term, because it will require a continuous effort to maintain and disseminate all these varieties (see Section 4.5, on Roles and responsibilities of partners).

For the process of priority setting, we have to consider which form and degree of diversity—and of which material—will be required to reach the diversity-related goals of the programme, and how important it is that the seed can be easily reproduced and re-used by the farmers. The latter point is discussed in more detail below.

FIGURE 4.4
Formal and informal dimensions of seed systems and how they may interact; varieties from the formal system may enter the local systems and vice versa



Source: Sperling and Christinck, 2005.

Variety types and farmers' access to seed

The seed channels farmers use for sourcing their seed are normally grouped into two broad seed systems: the formal and the informal seed systems. The latter is also sometimes termed the local, traditional or farmer seed system (see Figure 4.4).

The formal seed system involves a chain of activities that lead to clear products: i.e. certified seed of verified varieties. Thus, the chain usually starts with plant breeding in research institutions or commercial companies, and results in varieties or hybrids intended for formal variety release. Formal regulations aim to maintain varietal identity and purity, as well as to guarantee physical, physiological and sanitary quality. Seed marketing takes place through officially recognized seed outlets, either commercially, or via national agricultural research systems (Louwaars, 1994).

The informal system embraces most of the ways in which farmers themselves produce, disseminate and obtain seed: directly from their own harvest; through barter among friends, neighbours and relatives; and through local grain markets or traders. The same general steps take place in the informal system as in the formal, but they take place as integral parts of farmers' routine grain production rather than as separate activities. Also, rather than be monitored or controlled by government regulations, informal seed sector production is guided by local technical knowledge and standards, and by local social structures and norms, including market forces (McGuire, 2001). Varieties may be landraces or mixed

races, or improved varieties that have made their way into the local system.

Perhaps because of their ability to meet local needs and preferences, informal channels provide most of the seed that small farmers use: it is estimated that somewhere between 80 and 90 percent of total seed sown originates from the informal system, although this varies a lot between different countries and regions, as well as for different crops. A formal seed system does not exist in practice for many local crops or varieties of minor economic importance, whereas it is particularly important in regions where hybrid maize is grown. The relative importance of the formal and informal seed systems also much depends on the seed legislation of the respective country. Very restrictive seed laws have practically abolished the informal seed system in some countries, whereas in others the legislative framework allows for the co-existence of both systems.

Professional plant breeders are usually members of formal institutions (public or private), so that formal channels of seed production and dissemination are the 'normal' route through which newly developed varieties find their way to farmers' fields. However, the formal and the informal systems have both comparative advantages and disadvantages for variety diffusion, and often address different client groups. Considering these differences could form part of an active strategy for effective variety diffusion in relation to the goals of the breeding programme. For example, the informal seed system has various advantages for poor farmers, as the seed price is usually lower and the modes of payment flexible. If poor farmers' access to new varieties is a goal of the breeding programme, variety diffusion through the informal system could be a good option for reaching this goal. At the same time, the informal system often builds on traditional rules and forms of cooperation in village communities, including cooperation among different wealth and ethnic groups. Thus, detailed knowledge of the seed systems and how they are related to different groups of farmers is required for developing such strategies (Sperling and Christinck, 2005).

The type of variety that will be developed, and how it can be reproduced and maintained by farmers, is thus a very important consideration for a breeding programme, particularly in situations where the formal system alone cannot serve the target groups of farmers.

4.5 ROLES AND RESPONSIBILITIES OF PARTNERS

4.5.1 Cooperation between different organizations and stakeholders

Plant breeding is increasingly being done as a partnership among different stakeholders: individuals, groups, organizations who share an interest in using and improving crops. It is thus clear that the discussion about roles and responsibilities of the different partners is at the heart of such plant breeding projects, and is thus a critical issue in the priority-setting process.

The 'history' of a project (who took the initiative and for what interest?) appears to play an important role in this regard. It makes a difference whether one organization initiated the project and organized the major part of the resources, and then sought potential partners, or whether it was a joint initiative from the outset. The present structure of international agricultural research, particularly with regard to funding and accountability, potentially poses problems for cooperative research that involves very different types of institutions. This is due to the large differences between organizations regarding

their access to external funding, and the fact that the institution that successfully acquires funds is usually alone accountable towards the donors, which often impedes a real sharing of project responsibilities among the partners (Kolanoski, 2003).

Notwithstanding, for the process of priority setting, it appears recommendable to look deeper into the key skills and resources (material and non-material) each partner or partner organization has to offer for reaching the identified project goals, for example, with regard to several issues:

- Overall project management, including decision-making processes, monitoring and evaluation, reporting, public relations work at different levels, fund acquisition and management.
- Planning and implementation of practical project activities, such as trial management and data analysis, or seed production and dissemination.
- Training and skill-building activities.

On this basis, contracts between the various institutions could be negotiated, which include tasks and duties with regard to the project, as well as the distribution of funds and resources among the partners. Furthermore, a pre-agreed procedure for mediation or a conciliation board should be foreseen in view of future cases of disagreement that might crop up between the partners.

4.5.2 Cooperation between farmers and scientists

In projects initiated by formal-sector breeding programmes, which are mostly concerned with the traditional goals of breeding programmes, such as productivity increases and possibly changes in policies for variety release or seed diffusion, most of the decision-making about the project tends to be initially in the hands of the scientists. The farmers often play a rather more consultative role, giving input into variety evaluation, prioritization of selection criteria, and the necessary insights required for focusing the project. However, as partners gain experience, and the scale at which the project operates increases, projects tend to develop towards a strengthened role for farmers or their organizations, especially in terms of selection decisions and variety evaluation.

If farmers, especially a farmer organization, initiate a plant breeding project, it tends to be clear that they seek specific support or input from scientists to find solutions to problems already well identified. In addition to specific technical support, scientists can make contributions to building farmers' skills with respect to obtaining new germplasm; crop biology or physiology; specific plant breeding activities, such as crossing; variety evaluation; and interpretation of results. In such situations, it is clear that the role of scientists is primarily a consultative one, while key decisions are taken by the farmers or their organizations.

In situations where farmers are not well organized, but project partners have identified farmer empowerment and skill building as a project goal, the project may invest major resources in the establishment of farmer organizations, committees or groups, which can then manage more of the key breeding activities, and over time become the primary decision-makers, as their skills and organizations grow. In such a scenario, the role of the researchers may change considerably over time, especially in terms of the management of trials, such as decisions about which materials to continue with or to abandon, or which priorities for selection to add to the project. Usually these changes are also accompanied by a change in the scale of the project. There

is thus an increase in not only the skills of the farmers, but also in the number of farmers and of villages, and thus possibly the number of crops, target environments and priorities for selection.

In any case, a reflection on the different approaches and skills of farmers and researchers could be a valuable basis for priority setting with regard to roles and responsibilities of partners in a breeding project. Farmer experimentation is in various respects different from the experimental designs usually applied by scientists, and has been described by a number of authors (Johnson, 1972; ILEIA, 2000; Leeuwis, 2004; Reijntjes and Waters-Bayer, 2001; Saad, 2002). Respecting and learning from farmer's informal experimentation and evaluation approaches could lead to valuable insights and innovations, and could thus be assigned a role of its own in a participatory breeding project.

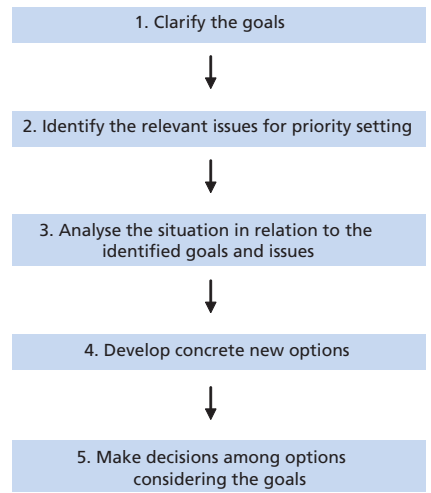
4.5.3 Decentralized breeding programmes

Breeding programmes that aim at exploiting local adaptation or increasing diversity in farmers' fields usually have to be organized in a strongly decentralized manner, as a number of varieties will have to be tested, multiplied and distributed among a limited number of users. In such cases, the responsibilities should also be shared from the outset to ensure the sustainability of such activities. Skill building, training and institutional development may be important elements in such projects, and could support farmers to manage locally preferred varieties by themselves.

4.6 PRACTICAL METHODS FOR PRIORITY SETTING

Priority setting for plant breeding programmes is, as such, not much different from other situations, and includes a number of steps (Figure 4.5).

FIGURE 4.5
Steps for priority setting in a plant breeding programme



Before examining some practical tools, we will briefly refer to each of the aforementioned steps.

4.6.1 Clarifying goals

As indicated earlier in this chapter, plant breeding programmes can have a variety of goals, of very different natures. It is important that all the options are discussed with the partners, and that a common vision is achieved for each project and for the programme as a whole. It is important that discussions about the goals are held regularly to ensure that the goals remain relevant, and that they remain clear, evident and important to all partners involved in the programme.

4.6.2 Identify the relevant issues for priority setting

The critical issues for priority setting in a plant breeding programme have been

outlined in the first part of this chapter (see Figure 4.1). All these issues need to be addressed by any plant breeding programme, but there may not always be viable alternatives to chose from. Besides, the goals of a breeding programme can change over time, reflecting the particular context or situation; thus, priorities need to be reviewed regularly.

For the purpose of identifying relevant options for the key issues, it may be helpful to examine the chances of success with regard to each of the goals. This could be done during a planning workshop, or also in the form of an e-mail discussion for those partners who are using this communication technology. Furthermore, it is likely that new options and insights emerge in the course of the practical project activities. Therefore, the process of priority setting should be implemented in such a way that insights and challenges can be addressed at regular intervals, and then be integrated into previous concepts.

4.6.3 Situation analysis

Realistic new options or technologies require a good knowledge of the situation under which they are intended to function, including the needs and preferences of the potential users. Client-orientation is a key concept in the general economy, and increasingly also in plant breeding (Witcombe *et al*., 2005). In the past, client-orientation was sometimes under-developed in plant breeding, particularly as far as resource-poor farmers in marginal areas were concerned. A basic understanding of the complexity of farming systems in such situations, as well as their dependency on environmental adaptation and biodiversity, has now been developing, mainly since the mid-1990s.

The situation analysis for a plant breeding programme should focus on those issues required to effectively reach the goals of the breeding programme. In general, it will have to include the following issues:

- agro-ecological conditions;
- socio-economic conditions, including marketing of crop-based products;
- the farming system, actual processes of change and main limitations;
- farmers' use of varieties and their seed management;
- seed system analysis; and
- specific varietal needs and preferences of the target group(s).

The situation analysis could include the following steps:

1. Review secondary sources.
2. Consult local experts, key people with good knowledge of the potential target area(s).
3. Visit potential target areas and consult farmers belonging to different social and wealth groups.
4. Structure and compile the information for further planning.

Experience gained in a number of PPB projects has shown that participatory communication tools, such as semi-structured or informal interviews, focus-group discussions, wealth ranking, transect walks, time lines, mapping, classification and ranking exercises, can be extremely useful for providing a good basis for further planning. The particular strength of such communication tools is that they facilitate direct dialogue between farmers and researchers, and can help to develop a common understanding of the situation, as well as of the main constraints and needs. Practical guidelines for conducting such a situation analysis, particularly for plant breeding projects, have been suggested by Christinck, Weltzien and Hoffmann (2005). Furthermore, much inspiration can be gained from general guides and

BOX 4.3

Web sites on participatory research methods

Sources of information and training materials are listed below. We concentrate here on those publications that are available via the Internet, often for free download.

1. The Web sites of FAO (www.fao.org) and the World Bank (www.worldbank.org) contain sections on publications for download and/or purchase (search for "participation" or "PRA").
2. Further publications may be found via the online bookshop of UNEP (United Nations Environment Programme) www.earthprint.com in the section on Participation and training.
3. An introductory guide to participatory learning approaches can be downloaded free of cost from the GTZ homepage: Schönhuth, M. & Kievelitz, U. 1994. Participatory Learning Approaches. Rapid rural appraisal, Participatory Appraisal – an introductory guide. http://www2.gtz.de/dokumente/bib/95-0930.pdf Other language versions (Spanish, French) are available upon request. More specific publications on participatory research and learning are accessible for download from: http://www.gtz.de/de/themen/uebergreifende-themen/partizipation/15201.htm (Accessed 12 September 2008).
4. Participatory Learning and Action (formerly PLA Notes) is a series on Participatory Learning and Action (Methods and Approaches), accessible through the IIED homepage (International Institute for Environment and Development, London, UK): http://www.iied.org/NR/agbioliv/pla_notes/about.html#a (Accessed 12 September 2008).
5. The Programme for Participatory Research and Gender Analysis (PRGA) has a Web site with a series of publications and resources, including a listing of cases for participatory plant breeding. (www.prgaprogramme.org)
6. Reading University, UK, maintains a Web site with training materials and resources focusing on the statistical analysis of data from participatory research activities: http://www.reading.ac.uk/ssc/workareas/participation.html (Accessed 12 September 2008).

publications on participatory research (see Box 4.3).

The use of qualitative social science methods for conducting studies in plant breeding projects has long been debated. Plant breeders are used to working with large numbers of accessions and observations on various trial sites, so that statistical data analysis is a standard method in this field of research. However, results from informal qualitative research are not necessarily less precise (only there are no numerical estimates of how precise). For many purposes in a plant breeding programme, and particularly in the initial phase, the main focus would be to initiate dialogue and identify potential partners. Often, it is possible to start with rather informal and qualitative research methods, in order to identify the main issues of relevance, and to use this knowledge later for more formal studies, if required. There are also increasing efforts to combine qualitative with quantitative, and informal with more

formal methods (Bellon and Reeves, 2002; Abbeyasekera, 2002).

4.6.4 Develop concrete new options

Developing new options for varieties requires creativity and good knowledge of the conditions under which a new variety will have to 'function'. It also requires good knowledge of the available diversity of the crop. Similarly, it may require detailed understanding of options for new crop uses, and for marketing of crop products, possibly new ones. Traditionally, plant breeders have done this based on their own understanding of the farmers' reality, especially as many of the early private plant breeders were farmers themselves. Nowadays, when plant breeders work on a national, regional or international scale, the development of new options for variety development, and seed distribution requires working creatively with farmers and other project partners from various institutions and disciplines. This is usually a continuing process, and thus the project or programme should be organized in such a way that regular reviews of alternative new options can take place.

4.6.5 Making decisions among various options considering the goals

Making choices between the different options needs to be forward looking, based on the identified project goals, and on chances for success. Different stakeholders and partners will have different perspectives, and thus their choices and preferences for specific options will vary. Hence it is important that the process of making decisions among an array of options is transparent, and that the roles and responsibilities of the different partners in the decision-making process are agreed. Ranking exercises are ideal tools for taking decisions based on transparent criteria. Participants may make their decision first, and then explain the reasons for their choice. Implicit reasons can thus be made explicit and transparent. More refined tools, which can consider several criteria simultaneously, may be used once the key criteria are agreed.

4.6.6 Tools for farmer participation in the priority-setting process

In this last section, we present a series of tools that have been used successfully in one or more of the steps of the priority-setting process outlined above. Some may be used only for one specific step in the decision-making process; others may apply to several of the steps. Many of the tools have been successfully used with farmers for the identification of critical selection criteria. The tools we choose to describe are primarily those that can be used with a wide variety of partners, specifically with farmers, but also with those who may have very little time, may not be literate, but may have a profound knowledge of their culture and crop related issues. Many of the tools are described in more detail and with more examples in other sources, sometimes in other contexts. Some good source materials are cited and listed. In most instances, one would apply not only a single tool, but several; it is advisable to vary the tools for different steps of the priority-setting process, and also for the purpose of verifying and increasing the reliability of previous results and hypotheses.

Facilitated discussions on goals, issues and criteria

Invite all relevant project partners to a meeting on discussing goals for a new plant breeding programme. As the outcomes will possibly depend on the circle of persons invited, the invitation list should be carefully thought out. Furthermore, particularly if

farmers are involved, the language, the general 'setting' and the working style (are all participants literate?) should be considered with awareness.

Depending on the number of participants, there are various options for facilitating such a meeting. One option would be that the participants from each organization are asked to prepare a short presentation, which would include a sort of problem analysis based on their own experience and viewpoints, and should propose goals and priorities. After the presentation, the main goals mentioned in the presentation would be documented on a board. In this manner, there would be a preliminary list of goals at the end, which could then be further discussed.

Another way would be to start with a 'brain-storming session' or open discussion on goals, and to document the proposed goals on a board for further discussion.

There should then be time to discuss these goals in more detail and clarify what they imply. Very often, it helps if the participants are asked what kind of indicators they would suggest as a 'measurement' of whether the future project activities would be successful or not in reaching these goals. Such indicators could thus also be useful for future monitoring and evaluation meetings.

It is of particular importance to identify potentially conflicting goals, or utopian goals. In such cases, the group could try to weigh up different goals, or to make utopian goals more realistic and situation-specific. In general, it is of course much easier to reach a few clear goals with high priority on the agenda of all participants, than a long list of potentially conflicting goals. At the same time, the discussion of goals can anticipate many problems that might occur in the course of a plant breeding programme, particularly if many partners are involved.

The meeting could then finish by prioritizing the suggested goals, such as through a simple ranking or scoring exercise (see below).

In any case, such discussions on goals should be regarded as preliminary results. Many goals are not easily expressed and are closely related to individual or culture-specific values. Moreover, goals may evolve in the course of the project activities. It is thus recommended that this discussion be repeated later, for example after completing the situation analysis (see Section 4.6.3, above), and particularly in view of the question of whether the goals are really relevant for the target group. Regular discussions on goals and indicators, for example at the beginning of each new working phase, or in a general planning meeting, can be rewarding if a good facilitator helps to ensure productive outcomes.

SWOT analysis

A discussion about the overall goals and more specific priorities involving key actors or stakeholders can be structured in the format of an analysis of the present situation of the crop under discussion and the development of future varietal options. A strategic planning tool for this type of analysis is SWOT analysis, a structured discussion on **S**trengths, **W**eaknesses, **O**pportunities and **T**hreats. This discussion could be held as part of a project planning workshop, for example on the topic: 'Farmers' groundnut varieties for the dry areas of Senegal', or any other crop and region.

The participants, either individually or in small groups, are first asked to think about the strengths of the situation under discussion. The results should be documented on a board or piece of paper

(for later presentation to the whole group). In the following steps, the participants also discuss weaknesses, opportunities and threats. The results should be documented visually on a board, and could then serve as a starting point for discussion on goals and priorities of a breeding programme (see also Weltzien, 2005).

Recurrent feedback discussions

Successful project work depends on good interaction between partners, e.g. researchers from various institutions, farmers, and extension or NGO personnel. Feedback discussions during which the different partners openly exchange their views and experiences with specific project activities should be held at regular intervals. These discussions about what worked well, or which problems or opportunities arose, are the basis for reviewing the project priorities in an evolving partnership between very different types of organization. While there may not necessarily be a fixed framework for such discussions, they are instrumental in refining project priorities and in the evolution of the overall goals of a project and a partnership. Participatory Monitoring and Evaluation (PM&E) would be a more 'institutionalized' way of conducting such feedback discussions (Germann, Gohl and Schwarz, 1996).

Simple scoring exercises

If you wish to set priorities among a number of possible goals, criteria, problems or issues in a formal way, simple scoring exercises can be applied. This requires that a tentative list of goals and criteria is already established.

These goals should be written on a board or be represented visually in some form (graphically or as text). All participants get a predefined number of counters, such as pebbles, paper pieces, adhesive dots, etc., and are asked to put their counter next to those goals with the highest priority for them. The goals should be well understood for this exercise, and the rules explained carefully. Generally, each participant should have fewer counters than goals, so that a real decision has to be taken. It should be clarified whether it is allowed to assemble all counters at one goal, the one perceived to be more important than any other, or if only one counter can be placed for each goal. In this manner, you will obtain a clear result within a relatively short time—a result on which further discussions can be based.

Ideal variety

Invite a small group of participants, preferably 2 to 4, with whom you have already discussed variety trials or the importance of specific traits in particular. Larger groups could split up into separate working groups and later present their results to the whole group. Invite each participant to think about what a really good variety of the crop on which you are working could look like, referring to the previous discussions you have had. Focus group discussion, where different groups represent farmers with differing backgrounds, farming situations, gender, ethnic groups, etc., can reveal underlying differing needs.

Ask the participants to think about all the characters that a good variety of millet, cowpea, etc., should have, to be useful for them. The traits mentioned by the participants should be written on cards, or the participants should find symbols for visual representation; the cards should then be placed vertically in a column. Make sure that everybody contributes and that all the important traits are mentioned. In the course of the exercise, you may also suggest some trait(s) if you are particularly

interested in sparking off a discussion on the relative importance of some new traits. Once all the traits have been identified, you can then ask the farmers to discuss the importance of the trait for a new variety that would be better than the existing ones. To indicate the level of importance of each trait the farmers could distribute a fixed total number of tokens between the traits they (and you) have mentioned. The more important a trait, the more tokens it receives. Traits that are not required should get no tokens, and can be eliminated.

It is best to facilitate this discussion in such a way that the participants primarily discuss among themselves about each trait; for example, how early the ideal variety should be, or how much grain yield in relation to stover yield they think would be useful. The difficulty is to try to keep the discussion within the realm of biological reality, i.e. **not** only grain yield, increased 10-fold with half the growth duration of existing varieties.

Create scenarios

Scenarios can be used to find out whether certain concrete new options are attractive for the target group(s) of farmers. This approach is particularly useful in the case of complex or interrelated trait combinations. For this purpose, we need seed and plant material in which these new trait combinations are already expressed (i.e. exotic or experimental varieties).

By simulating a situation in which farmers have to take a decision between various complex options, immediately followed by an interview about the reasons, then important criteria and trade-offs may be revealed. Furthermore, this is also a way to study whether and why people belonging to different groups take different decisions regarding the proposed options.

Scenarios are only useful if the farmers' reality is reasonably well understood. If the options or choices presented to farmers are not realistic, the responses cannot be expected to be realistic either.

Example 1: Seed shop exercise

The scenario is that the farmer who has no seed of this crop at the time of sowing enters into a seed shop and has to choose among a set of varieties with different properties.

For this purpose, seed of different varieties, local and introduced, is displayed in the 'shop', so that the farmers can see and touch the seed. Variety names, plant samples or drawings of the plant type can provide additional information. If you really plan to give the seed to the farmers after the exercise, small packages in sufficient number should be prepared.

The farmers are asked to enter the 'shop' one by one, take their decision and leave the 'shop'; an interview on the reasons for their choice will be conducted immediately after leaving the 'shop'.

The rules of the exercise should be made very clear at the beginning, particularly concerning questions such as whether the farmers will really get seed of the preferred variety, how much, at what time (in the 'shop' or afterwards) and from whom. Such rules potentially influence the result. They should be carefully considered beforehand and then announced very clearly to the participating farmers.

Example 2: Simulating plant selection in a 'field'

The scenario here is that a farmer selects plants from a 'field'. This is very close to the farmers' reality in most cases. A further advantage of this scenario is that many different traits, which may be relevant for the adaptation to specific conditions, different

uses or situations, will be included into the farmer's decision-making.

A small plot or grow-out of a variety mixture or broad-based population will be required, which shows variability with regard to all traits in which the farmers or the plant breeders are interested (known from previous exercises).

The participating farmers are invited individually or in groups to the 'field'. They are then asked to mark with a coloured ribbon or tag a certain number of plants that they would select for growing in their field. Alternatively, the farmers could be asked to cut the plants from the plot for further evaluation. Interviews on the choices taken by the participants could follow.

Simple ranking

If decisions have to be taken among few options (2 to 5), write the options on paper cards or represent them visually with photographs, drawings or real objects. The options and what they imply should be very clear to the participants. Ask a person or small group to put the cards or objects in an order of preference, starting with the best, the second best, third best, etc. Then ask for reasons and criteria used. A detailed description and training exercises can be found in Guerrero, Ashby and Gracia (1993).

Pair-wise ranking

This exercise works well with up to six items or options. The participants are asked to make pair comparisons, indicating which alternative is better, and why. This exercise often results in an exact description of the conditions under which the alternatives work well or otherwise. This exercise has proven very useful for discussions about selection criteria and farmers' preferences, and is explained in more detail by Weltzien and Christinck (2005).

Matrix ranking

Matrix ranking can provide more detailed insights into the advantages or disadvantages of various options. The ranking criteria have to be defined beforehand. Pair-wise ranking or the Ideal Variety exercises could be used to identify criteria for further discussion and variety evaluation. In a planning workshop, the different options or scenarios to be ranked can be related directly to the project goals, or to criteria that are related to the project goals; for example, if income generation through processing is one of the project goals, some of the ranking criteria could be concrete advantages for processing and marketing.

The matrix could be prepared on a large sheet of paper or on the ground. The visual or text representations of the different options to be ranked are usually placed vertically in a row, with the criteria or aspects in a horizontal row. The participants are then asked to rank all options for the first criterion by placing counters (adhesive markers if done on paper, otherwise pebbles, large seeds, etc.). There should be clear rules for placing counters (i.e. only one counter for the option that fulfils best this criterion; or a certain number of counters for the best, second best, etc.).

If you assign a number to each participant, and write the number on the counters used by this person, the result could be useful for further analysis (who preferred which option, and why). Thus matrix ranking needs some efforts for preparation, but can then deliver very detailed results, especially for identifying selection criteria, user groups and target growing conditions.

Scoring exercises

Scores are frequently used by breeders to assess newly-created varieties and breeding

lines. A similar approach can also be pursued with farmers.

Scores indicate a certain level of performance or expression of a trait. For example, the early vigour of varieties could be assessed using a score, where 5 indicates that a variety is extremely vigorous, 4 = very vigorous, 3 = vigorous, 2 = less vigorous and 1 = not vigorous, or weak. Thus scoring applies a fixed scale, as a tool for assessing potentially a large number of new varieties or other options.

There is a fundamental difference between scores and ranks, which can have far-reaching implications. For example, ranking puts varieties in the order of performance or expression of a specific trait. The best variety could actually have a fairly poor performance, if all the other varieties are still worse. The differences between varieties could be very small, but they may lead to different ranks. Ranks do not have an underlying scale, and thus quantitative analysis is more difficult. Ranking can only be done meaningfully with a small set of varieties (not more than seven) (Coe, 2002; Weltzien and Christinck, 2005).

Discussions on the reasons for giving a particular score to a variety will reveal the underlying criteria. It is furthermore possible to compare the scores given by different groups of farmers (gender groups, people from different villages, etc.).

Practically, scoring exercises can be realized in the field in various ways. Literate participants can enter scores (= numbers) in a previously prepared evaluation form. Alternatively, one can use counters (stones, pebbles, paper pieces), which have to be put into a basket, box or bag near the scored plot. More detailed descriptions and examples can be found in Weltzien and Christinck (2005).

Discussions with farmers about their scoring will lead to a better understanding of selection criteria, preferences of specific user groups or for target growing conditions, market demands, etc.

Other tools used for priority setting

The tools described above are explained in more detail in various training manuals and handbooks (Box 4.4) for farmer participatory rural appraisals. Economists tend to use

BOX 4.4

Training materials and books on participatory research methodologies in plant breeding projects

1. Bellon, M.R. & Reeves, J. 2002. *Quantitative analysis of data from participatory methods in plant breeding.* Mexico, CIMMYT.
2. Christinck, A., Weltzien E. & Hoffmann, V. 2005. *Setting breeding objectives and developing seed systems with farmers. A handbook for practical use in participatory plant breeding projects.* Margraf Publishers, Weikersheim, Germany, and CTA, Wageningen, Netherlands.
3. IPRA & CIAT. 1991. *Farmer evaluations of technology: Methodology for open-ended evaluation.* Instructional Unit No. 1. IPRA, CIAT, Cali, Colombia.
4. Guerrero, M.P., Ashby, J.A. & Gracia, T. 1993. *Farmer evaluations of technology: Preference ranking.* Instructional Unit No. 2. IPRA, CIAT, Cali, Colombia.
5. Cleveland, D.A. & Soleri, D. 2002. *Farmers, scientists and plant breeding: Integrating knowledge and practice.* Wallingford, UK, CABI.

other tools, such as Decision Trees, Grid Analysis or Hedonic Pricing Models, for priority setting and the identification of specific selection criteria. These tools have rarely been applied specifically to plant breeding programmes, with the important exception of the hedonic pricing model, which has been used in a number of instances (e.g. Dalton, 2004; Faye *et al*., 2004). These quantitative analytical tools can also be used to analyse data from specifically set up scenarios, or from ranking or scoring exercises. More examples for combining qualitative and quantitative tools can be found in Bellon and Reeves (2002) or Barahona and Levy (2002).

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Christinck, A., vom Brocke, K. & Weltzien, E. 2000. What is a variety: Investigating farmers' concepts as a base for participatory plant breeding in Rajasthan, India. *In* International Agricultural Research. A contribution to crisis prevention. Conference on Tropical Agriculture and Forestry, University of Hohenheim, Stuttgart, Germany, 11–12 October 2000 (available at http://www.prgaprogram.org/ppb_resources/ppb_christinck_00.pdf)

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CHAPTER 5

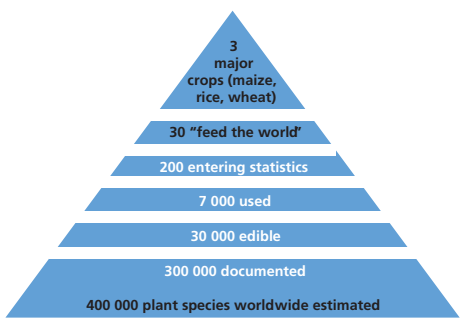
Methodologies for generating variability. Part 1: Use of genetic resources in plant breeding

Bettina I.G. Haussmann and Heiko K. Parzies

5.1 INTRODUCTION

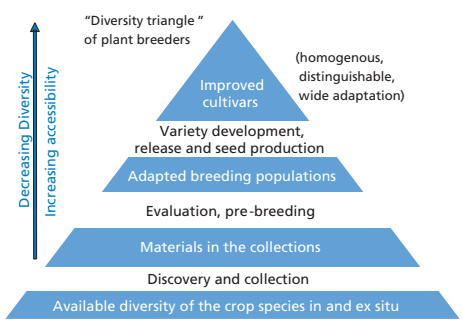
Both inter- and intraspecific diversity is declining in our present agricultural systems. Out of an estimated total of 30 000 (FAO, 1996a) to 50 000 (Sánchez-Monge, 2002) edible plant species, only 30 "feed the world", with the three major crops being maize (*Zea mays*), wheat (*Triticum aestivum*) and rice (*Oryza sativa*) (FAO, 1996a; Figure 5.1). At the intraspecific level, plant breeding contributes to a diminution of diversity through development of narrow, elite breeding populations, selection of the 'best' genotypes, development of homogeneous cultivars, and promotion of a few, widely adapted varieties (Figure 5.2).

FIGURE 5.1
Use of crop species diversity in agriculture



Source: FAO, 1996

FIGURE 5.2
The diversity triangle of plant breeders



Source: FAO, 1996

However, the decline of inter- and intraspecific genetic variability among and within cultivated crop species bears with it several risks, including epidemics of pests and diseases due to greater genetic vulnerability; lack of adaptation to climate-change-related stresses; lack of genetic variation for specific quality traits; and reaching performance plateaus. A more efficient use of plant genetic diversity is therefore a prerequisite for meeting the challenges of development, food security and poverty alleviation (FAO, 1996b). Concrete aims of using plant genetic resources (PGR) in crop improvement are:

- to develop cultivars that are specifically adapted to abiotic or biotic stresses;
- to assure sustainable production in high-yielding environments through reduced application of agrochemicals and increased nutrient and water efficiency; and
- to open production alternatives for farmers through development of industrial, energy or pharmaceutical crops.

Methods of using PGR in crop improvement have recently been reviewed (Haussmann *et al.*, 2004). Major points will be summarized in this chapter, but for details and more examples, the reader is referred to the full review article. After the generalities concerning use of plant genetic resources (PGR) in plant breeding, this chapter will also consider more specific aspects of using plant genetic resources in participatory plant breeding, such as management of diversified populations and their potential contribution to *in situ* PGR conservation; the use of landraces as genetic resources for adaptation to stress environments, climate variability and climate change; and to better serve farmer's and end-user's diverse needs.

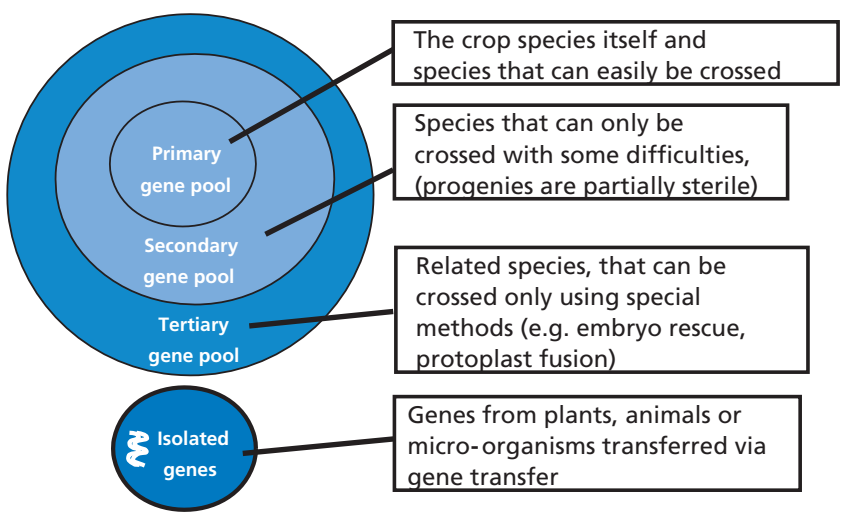
5.2 DEFINITION OF GENETIC RESOURCES FOR PLANT BREEDING

PGR can be defined as all materials that are available for modification of a cultivated plant species (Becker, 1993). PGR have also been considered as those materials that, without selection for adaptation to the target environment, do not have any immediate use (Hallauer and Miranda, 1981). According to the extended gene pool concept, genetic resources can be divided into primary gene pool; secondary gene pool; tertiary gene pool; and isolated genes (Harlan and de Wet, 1971; Becker, 1993; Figure 5.3). The primary gene pool consists of the crop species itself and other species that can be easily crossed with it. The secondary gene pool is composed of related species that are more difficult to cross with the target crop, i.e. where crossing is less successful (low percentage of viable kernels) and where crossing progenies are partially sterile. The tertiary gene pool consists of species that can only be used by employing special techniques, like embryo rescue or protoplast fusion. The fourth class of genetic resources, isolated genes, may derive from related or unrelated plant species, from animals or micro-organisms.

5.3 FACTS AND INFORMATION SOURCES

Worldwide, 1 308 gene banks are registered and conserve over 6.1 million accessions, including major crops, minor or neglected crop species, together with trees and wild plants. Of the 30 main crops, more than 3.6 million accessions are conserved *ex situ* (FAO, 1996a). Little information exists about documentation and availability of materials that are maintained *in situ*. Links to some of the most important organizations or networks dealing with PGR are listed in Box 5.1.

FIGURE 5.3
The extended gene pool concept for classification of PGR



Source: modified from Becker, 1993, and Harlan and de Wet, 1971.

BOX 5.1

Some important organizations and networks dealing with PGR.

- World Information and Early Warning System (WIEWS) on Plant Genetic Resources for Food and Agriculture (PGRFA) — http://apps3.fao.org/wiews/
- Consultative Group of International Agricultural Research (CGIAR) System-wide Information Network for Genetic Resources (SINGER) — www.singer.cgiar.org
- Bioversity International — www.bioversityinternational.org
- Germplasm Resources Information Network (GRIN) and the National Plant Germplasm System (NPGS) of the United States Department of Agriculture — www.ars-grin.gov/npgs/
- Mansfeld database — http://mansfeld.ipk-gatersleben.de/Mansfeld/
- The Information System on Genetic Resources (GENRES-International) — www.genres.de

5.4 DOCUMENTATION AND EVALUATION OF PGR

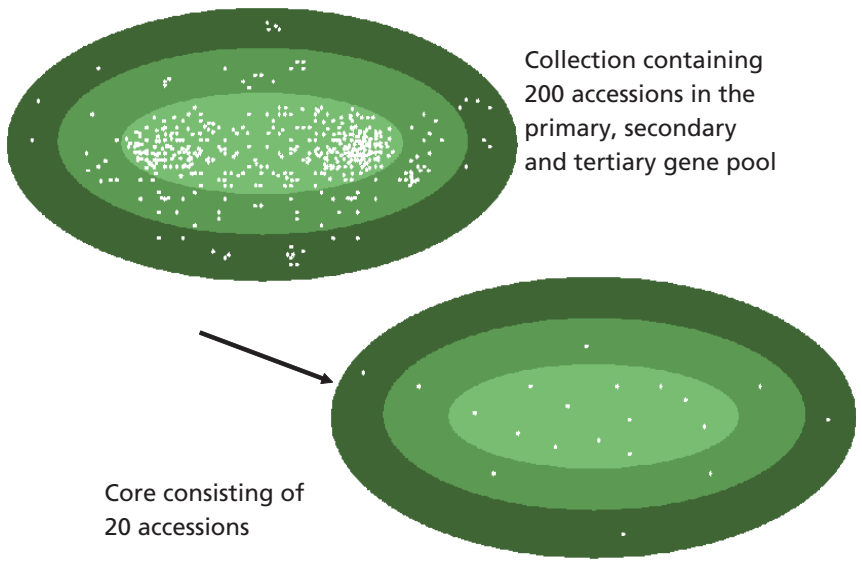
Gene bank accessions are described by passport and characterization data, and to a variable extent also by evaluation data. Passport data include serial number, taxonomic name, collection site, date of collection and donor institute. Additional notes can refer to seed viability, number and mode of regenerations or reproduction, and information about the distribution of the sample. Germplasm passport information exchange is facilitated by the internationally standardized list of multi-crop passport descriptors (FAO/IPGRI, 2001). Characterization data usually comprise scores for simple morphological traits like plant height, maturity date and thousand-seed weight. Evaluation data refer to agronomic traits like grain yield, grain quality, lodging and resistance to important pests and diseases as far as evaluated. Evaluation is a continuous process. Different people or institutions can be involved, including gene banks, breeders, pathologists or physiologists searching for or studying specific traits. Ideally, all data sets referring to an accession are stored in a central database and are made available to the public.

Systematic evaluation of germplasm conserved *ex situ* is facilitated through development of core collections. Initially, core collections were defined as a limited set of accessions representing, with a minimum of repetition, the genetic diversity of a crop species and its wild relatives (Frankel, 1984). In the context of an individual gene bank, a core collection consists of a limited number of the accessions of an existing collection, chosen to represent the genetic spectrum of the whole collection (Brown, 1995; Figure 5.4). Core collections render the evaluation process more efficient because repetition of similar entries is avoided (Hodgkin *et al.*, 1995; van Hintum *et al.*, 2000).

5.5 ACCESS TO PLANT GENETIC RESOURCES, EQUITABLE SHARING OF PROFITS AND BENEFITS, AND MATERIAL TRANSFER AGREEMENTS

The Convention on Biological Diversity (CBD) aims at the conservation and sustainable use of biological diversity, and an equitable sharing of profits and benefits generated by the use of genetic resources (www.cbd.int). One aim of the convention is to ensure recognition of the past, present and future contributions of farmers to the

FIGURE 5.4
The concept of core collection



conservation and development of genetic diversity (Swaminathan, 2002). To fulfil the convention, so called Material Transfer Agreements (MTAs) have been developed. The Standard MTA (SMTA, www.cgiar.org.cn/pdf/SMTA_English.pdf) protects the genetic resources of plant species listed in the Annex 1 of the International Treaty on Plant Genetic Resources in Food and Agriculture (www.fao.org/ag/cgrfa/itpgr.htm#text) against intellectual property rights and assures continuous and free availability. A special paragraph deals with the equal sharing of benefits (Figure 5.5).

MTAs from other institutions may refer to restricted plant materials, and in this case the user has to agree to use the material for research only; not to distribute or commercialize the plant material or derived materials; and to take all reasonable precautions to prevent unauthorized propagation of any of this material or derived plant materials.

5.6 METHODS OF USING GENETIC RESOURCES IN PLANT BREEDING

After identification and acquisition of potentially useful PGR, there are generally four ways of using those genetic resources in plant breeding (Simmonds, 1993; Cooper, Spillane and Hodgkin, 2001; Figure 5.6):

- introgression, which involves the transfer of one or few genes or gene complexes (chromosome segments) from a genetic resource into breeding materials;
- incorporation (also named genetic enhancement or base broadening) describes the development of new, genetically broad, adapted populations with a new range of quantitative variation and acceptable performance level;
- pre-breeding, which refers to more basic research activities with the goal of facilitating use of 'difficult' materials; and
- gene transfer.

Sometimes, the categories cannot be clearly separated one from another.

FIGURE 5.5
Some key clauses of the Standard Material Transfer Agreement (SMTA)

The Recipient may utilize and conserve the material for research, breeding or training purposes.

The Recipient shall not claim any intellectual property or other rights that limit the facilitated access to the Material provided under this Agreement, or its genetic parts or components.

In the case that the Recipient commercializes a product that is a Plant Genetic Resource for Food and Agriculture and that incorporates Material as referred to in Article 3 of this Agreement, and where such Product is not available without restriction to others for further research and breeding, the Recipient shall pay a fixed percentage of the sales of the commercialized product into the mechanism established by the Governing Body for this purpose, in accordance with Annex 2 to this Agreement. …

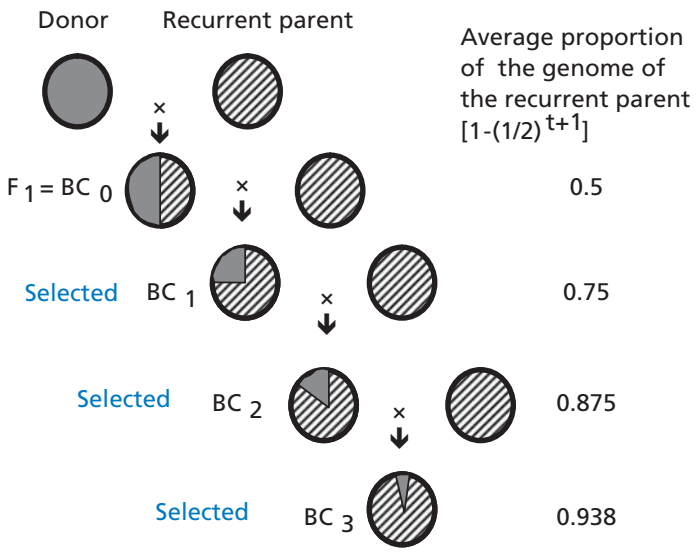
FIGURE 5.6
Overview over methods for using PGRs in plant breeding

Identification (out of 6.1 million accessions)	→	Phenotypic and molecular-genetic characterization, data management
Transfer of superior characteristics		
Introgression	→	Backcrossing of qualitative traits (possibly marker-assisted)
Incorporation ('Basebroadening')	→	Population improvement for quantitative traits (possibly marker-assisted)
'Pre-breeding'	→	Wide crosses
Gene transfer	→	Transformation

5.6.1 Introgression

Introgression aims at improving highly heritable qualitative traits that are governed by one or a few major genes or gene complexes. Traditionally, the classical backcrossing method is used to introgress traits like resistances or restorer genes from wild relatives (= the donor) into breeding materials (= the recurrent parent) (Figure 5.7). The method is particularly effective if the trait to be transferred is dominant. In the case of recessive inheritance, all backcross

FIGURE 5.7
Classical back-crossing for transfer of qualitative traits from a donor into a recipient



progenies need to be selfed in order to identify the carriers of the target allele, before the next backcross of the selected plants can take place.

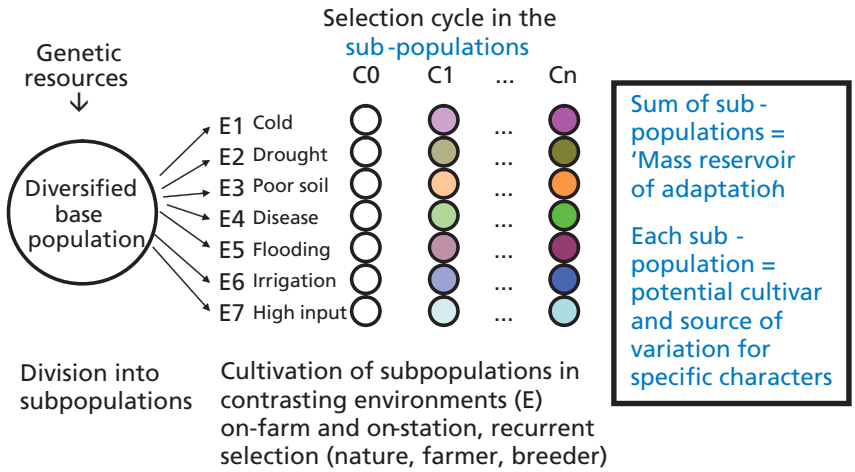
5.6.2 Incorporation

Incorporation, genetic enhancement or base broadening aim to increase the genetic variation for quantitative traits (i.e. traits that are due to many gene loci with small effects) in adapted genetic backgrounds. Various methods of population improvement can be used. The methods will vary depending on the crop species (self- or cross-pollinating) and the available time frame. Initially, selection may concentrate on adaptation traits that are highly heritable; performance traits are selected at a later stage. Diversity and recombination are maximized in the initial phase, with minimal selection intensities. According to the available time frame, two main categories can be distinguished:

- long-term development of synthetic or composite-cross populations and dynamic gene pool management; and
- short-term genetic enhancement to increase the actual variation in breeding populations.

To develop synthetic or composite-cross populations, a large number of accessions of different geographical origin and with maximal genetic diversity are crossed. The resulting population is divided into subpopulations (effective population size N>1000) and the subpopulations are grown for up to 30 generations in a number of different environments. This process is called dynamic gene pool management. At each site, recombination is promoted, and both natural selection and mild mass selection may contribute to adaptation of the individual subpopulations to the site-specific stresses or growing conditions. The sum of all subpopulations has been termed "mass reservoirs of genetic adaptability"

FIGURE 5.8
The concept of dynamic gene pool management



(Simmonds, 1993; Cooper, Spillane and Hodgkin, 2001) and is also understood as a means of *in situ* maintenance of PGR (Figure 5.8). Examples are the barley (*Hordeum vulgare*) composite cross developed at Davis, California, United States of America (Cooper, Spillane and Hodgkin, 2001), dynamic gene pool management in wheat (Goldringer *et al.*, 2001); pearl millet (*Pennisetum glaucum*) composite populations developed in Africa (Niangado, 2001); and the development of locally adapted 'farm cultivars' for ecological agriculture in Europe (Müller, 1989).

In the short term, genetic enhancement of breeding materials, genetic resources are selected for desirable agronomic traits and yield performance, but not for the highest degree of genetic diversity. They are intercrossed, recombined and then selected for adaptation to the target environment. To speed up the process, selected PGR may also be crossed with the breeding materials, and selection for yield traits carried out in the F_2 (50% exotic genome) or BC_1 (25% exotic) generation. The optimal percentage of the exotic genome of the genetic resource (100%, 50% or 25%) in a breeder's population depends on the overall objective; time available and finances; the level of adaptation of the genetic resource; and the yield difference between the genetic resource and the actual breeding population. Direct adaptation of the PGR takes usually longer than selection in F_2 or BC_1 (due to lack of adaptation of the PGR) but will result in materials that are genetically quite different from the actual breeding materials, which can be an advantage. Selection in BC_1 may be preferred over selection in an F_2 population if the PGR is highly unadapted to the target environment. At the same time, selection in the F_2 population is expected to reveal a higher genetic variance, a component of the expected gain from selection (Bridges and Gardner, 1987).

5.6.3 Pre-breeding and wide crosses

Pre-breeding includes basic research to achieve wide crosses, and activities that facilitate the use of exotic materials or wild relatives. It can refer to both qualitative and quantitative traits and the distinction between pre-breeding, introgression and incorporation is not always clear. The main objective is to provide breeders with more 'attractive' genetic resources that are easier to use, such as resistance sources in an acceptable genetic background; or inbreeding-tolerant forms of out-crossing species for hybrid breeding. An example of a very innovative use of wide crosses is the New Rice for Africa (NERICA) developed by the Africa Rice Center (WARDA, www.warda.org). Through crossing the African upland rice, *Oryza glaberrima,* with wetland Asian rice, *O. sativa*, and using embryo rescue and farmer-participatory variety selection, new rice cultivars were developed that combine positive characters (high grain yield and resistances to pests and diseases) of both rice species (www.warda.org/warda1/main/Achievements/nerica.htm).

5.6.4 Gene transfer

Gene transfer is independent of crossing barriers and may therefore increase the usable genetic variation of and beyond the tertiary gene pool. The principal steps for gene transfer from any species into cultivated crops are: gene isolation; gene cloning; gene transfer; and final expression studies in greenhouse and field trials across several generations of progeny. The details of gene transfer go beyond the scope of this chapter. Within the next 10 to 15 years, transformation research hopes to reach the following goals: controlled integration and stable expression of transferred genes; targeted manipulation of multigenic characters; efficient production of transgenes; transgenes, without or with harmless selection markers; and efficient transformation of cell organelles to ensure maternal inheritance, and thereby avoid unwanted horizontal gene transfer (Daniell, Khan and Allison, 2002). Classical examples of the use of gene transfer are the improvement of insect resistance through transfer of *bt* genes from *Bacillus thuringiensis* into crops like tobacco, tomato, maize, rice, cotton and soybean; the improvement of virus resistance through transfer of viral coat proteins in tomato and potato; and the creation of herbicide-resistant crops through transfer of bacterial or fungal genes into sugar beet, tomato and rape. There are also increasing efforts to improve stress tolerance of crops through transfer of genes for improved osmoregulation, heat shock proteins, phytohormone synthesis, and other traits from different organisms into cultivated plants. More information and numerous references on genetic engineering of stress tolerance can be found on the Web site www.plantstress.com.

5.7 UTILITY OF MOLECULAR MARKERS AND GENOME RESEARCH FOR USING GENETIC RESOURCES IN PLANT BREEDING

The utility of molecular markers and genome research in the context of using PGR for crop improvement include:

- diversity studies to distinguish genetically similar or distinct accessions, and to determine individual degrees of heterozygosity and heterogeneity within PGR populations;
- genetic mapping to identify markers in close proximity to genetic factors affecting quantitative trait loci (QTLs), followed by marker-assisted selection (MAS) of desired genotypes in segregating populations;

- exploitation of valuable QTLs from PGR by advanced backcross QTL analysis to combine QTL analysis with the development of superior genotypes or by marker-assisted, controlled introgression of PGR into breeding materials through the development of introgression libraries; and
- association studies to mine directly the allelic diversity of PGR collections and to identify those alleles that are beneficial for important agronomic traits.

5.7.1 Diversity assessment

For an efficient diversity assessment, molecular markers ideally need to be selectively neutral, highly polymorphic, co-dominant, well dispersed throughout the genome, and cost- and labour-efficient (Bretting and Widerlechner, 1995). Genetic markers complying with these requirements are protein markers (i.e. iso-enzymes) and DNA markers such as Restriction Fragment Length Polymorphisms (RFLPs) and Microsatellites or Simple Sequence Repeats (SSRs). Because the development of the latter two marker types requires prior knowledge of DNA sequences, a number of universal, dominant molecular marker types such as Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphisms (AFLPs) have also been employed in PGR diversity studies. However, the latter are not suitable for assessing factors such as mating behaviour or heterozygosity of the germplasm.

Generally, genetic diversity can be measured on three levels: in individual plants, within populations (intrapopulation) and between populations (interpopulation), while populations are considered as groups of randomly interbreeding individuals of one species. The diversity of individual plants is most commonly characterized in terms of the heterozygosity, i.e. the average number of heterozygous gene loci.

At the population level, protein markers and DNA markers are commonly used to calculate, among others, (i) allelic diversity or allelic richness (A; the mean number of alleles per locus); (ii) percentage of polymorphic loci (P; the mean proportion of polymorphic loci); (iii) Nei's average gene diversity (H_e; which denotes the probability that two randomly chosen alleles at a certain locus from a population are different. It is the generalized form of expected heterozygosity assuming Hardy-Weinberg Equilibrium and thus often abbreviated as H_e); and (iv) Shannon's index of diversity (H), which is widely used in ecology but also applied to population genetics (Lowe, Harris and Ashton, 2004).

With the employment of DNA point mutations, such as single nucleotide polymorphisms (SNPs) and small DNA Insertion/Deletions (InDels) as markers for diversity studies, a number of indices have been put forward for variants of a certain DNA sequence in a population. These are (i) the number of polymorphic (segregating) sites (S); (ii) total number of mutations (Eta); (iii) number of haplotypes (h); (iv) haplotype (gene) diversity (H_d); (v) nucleotide diversity (Pi; the average number of nucleotide differences per site between two sequences; Nei, 1987); (vi) nucleotide diversity (Pi (JC); the average number of nucleotide substitutions per site between two sequences (Lynch and Crease, 1990, cited by Rozas *et al.*, 2003); (vii) Watterson estimator Theta (Watterson, 1975); on a base-pair basis it can be interpreted as $4N\mu$ for an autosomal gene of a diploid organism, where N and μ are the effective population size and the mutation rate per nucleotide site per generation, respectively); and (viii) average

number of nucleotide differences (k). It seems noteworthy that indices of nucleotide diversity allow implications that go beyond quantifying the diversity of a population. For instance, the Watterson estimator Theta allows one to infer the effect of selection on a certain locus. However, detailed description of these indices is beyond the scope of this chapter. For further reading refer to Rozas *et al*. (2003).

Diversity between populations is commonly illustrated through graphical presentation of results of multivariate methods (cluster analyses) in the form of dendrograms (e.g. based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) or Neighbour-Joining algorithms) and two- or three-dimensional plots (e.g. Principal Coordinate Analyses). The bases for all cluster analyses are pairwise dissimilarity coefficients (distance/similarity measures) between all respective populations of a study. Some important dissimilarity coefficients for co-dominant marker data are (i) Euclidean Distance; (ii) Modified Rogers' Distance; (iii) Nei's genetic distance; and (iv) Reynolds' dissimilarity (which is based on the co-ancestry coefficient).

Some important similarity coefficients for dominant marker data are (i) Simple matching; (ii) Jaccard (1908, cited by Reif, Melchinger and Frisch, 2005); and (iii) Dice (1945, cited by Reif, Melchinger and Frisch, 2005). A comprehensive account of the dissimilarity indices mentioned here is given by Reif, Melchinger and Frisch (2005) and also by Mohammadi and Prasanna (2003). Considering the partitioning of diversity within and between populations, Wright's Fixation index (F_{ST}), which is calculated from allele frequencies, plays an important role in diversity studies (Lowe, Harris and Ashton, 2004). Besides measuring the partitioning of diversity between and within populations, it can be interpreted as a measure of differentiation between subpopulations, and also as the reduction of heterozygosity of subpopulations due to random genetic drift. In this respect, F_{ST} offers the possibility to calculate gene flow (N_m) between populations according to the formula $N_m = (1 - F_{ST}) / 4F_{ST}$, which can be interpreted as the number of migrants between populations per generation. As the latter indices only apply to co-dominant marker types, Excoffier, Smouse and Quattro (1992) developed a variance-based technique—analysis of molecular variance (AMOVA)—to calculate analogous indices to F_{ST}, which they called Phi_{ST}. AMOVA can also be used to characterize the diversity of populations in terms of variances regardless of the marker type.

It seems noteworthy that comparing data achieved with different molecular marker types, or even measured at different marker loci of the same type, is ambiguous, as diversity measures are relative rather than absolute (Ennos, 1996). For this reason, some authors give diversity indices for a certain marker locus as polymorphism information content (PIC), which provides an estimate of the discriminatory power of a locus (Botstein *et al.*, 1980). The use of PIC values allows the direct comparison of population diversity from different studies, provided that the same marker loci have been used.

A different objective of molecular diversity studies is heterotic grouping of genotypes suitable for hybrid breeding approaches. The principle behind this approach is the search for a correlation between genetic distance and heterosis, i.e. the more distant two genotypes of a crop species are genetically, the more heterozygosity, and therefore heterosis, can

be expected in the hybrid resulting from a cross between them (Melchinger, Coors and Pandey, 1999; Reif *et al*., 2003a, b). Yet, the effect on heterosis and hybrid performance needs to be distinguished, since high heterosis does not necessarily mean high hybrid yield. Recent studies have shown that the correlation between diversity measures and hybrid performance gets stronger when the markers used for diversity assessment are linked to performance QTLs, rather than from using neutral markers (Vuylsteke, 1999; Vuylsteke, Kuiper and Stam, 2000; Jordan *et al.*, 2003).

5.7.2 Genetic mapping and marker-assisted selection

Marker-assisted selection (MAS) can help (i) to select individuals carrying molecular markers that are linked to the trait of interest, instead of performing extensive phenotypic tests (foreground selection); and (ii) to reduce undesired parts of the donor genome, including the linkage drag (background selection). Foreground selection requires a tight linkage between the trait of interest and its flanking markers for which one is selecting. Background selection necessitates genotyping with a larger number of markers, which cover the whole genome.

MAS has proven efficient for the transfer of simply inherited qualitative traits from genetic resources into elite materials using backcrossing procedures. It is particularly useful for traits that are recessive, that can be assessed only after flowering or that are very difficult and expensive to assess. By using a combination of foreground and background selection, the transfer of a monogenic trait from a genetic resource into a breeding line may be completed within three to four generations, instead of the usual six generations of classical backcrossing with the same proportion of the recurrent parent genome (Ragot *et al.*, 1995; Frisch, Bohn and Melchinger, 1999).

MAS for multigenic, quantitative traits at first requires the identification of the genomic regions (QTLs) that affect the trait of interest. In classical QTL mapping, a segregating population (e.g. F_2, F_3 or recombinant inbred population) is developed from two inbred lines. This mapping population is evaluated for the trait(s) of interest. Simultaneously, the population is genotyped with a number of markers and a genetic map is constructed from the marker data. In the final QTL analysis, data is analysed for co-segregation of particular markers with the trait of interest. QTL analysis is then followed by transfer of favourable QTL alleles into elite materials via pure MAS or MAS combined with phenotypic selection.

However, for complex, quantitative traits, the efficiency of QTL mapping and MAS is contested. There are a number of risks that can render MAS inefficient. For example, there may be no selection gain because of: unreliable QTL estimates (too few QTLs, with highly over-estimated effects); QTLs not being expressed in new genetic backgrounds; recombination between marker and QTL; unfavourable alleles of other genes linked to good QTL alleles; or too-high costs for marker analyses. It is therefore essential to use large mapping populations; genotype the mapping population with good genome coverage; assess phenotypic values in multi-environment field trials; cross-validate the gained data; verify QTL effects, using independent population samples, near-isogenic lines or different genetic backgrounds; ensure close linkage between marker and QTL, and verify the linkage by a phenotypic test in all 3 or 4 generations;

increase the marker density around the QTL to allow reduction of the linkage drag; and to optimize individual procedures while taking into account economic parameters. For quantitative traits, where many loci of minor effects are responsible, it is very difficult to obtain reliable, unbiased QTL estimates (e.g. Beavis, 1998; Melchinger, Utz and Schoen, 1998; Utz, Melchinger and Schön, 2000). Prospects for MAS are therefore more promising for traits that are determined by few QTLs with large effects (Melchinger, 1990).

5.7.3 Advanced backcross QTL analysis and introgression libraries

QTL analysis can also be performed in backcross generations derived from crosses of exotic PGR with elite materials. The Advanced Backcross QTL Analysis (AB-QTL; Tanksley and Nelson, 1996) combines QTL analysis with the development of superior genotypes and has been shown to be particularly useful for a trait transfer from poorly adapted germplasm. AB-QTL is therefore of special importance in the use of PGR for crop improvement. The starting point is a segregating generation of a cross between an exotic parent and an elite line that is analysed with as many molecular markers as possible. QTL mapping procedure is delayed until one of the advanced backcross generations ($\geq BC_2$) when lines or testcrosses are evaluated across environments.

To date, the AB-QTL strategy has been applied in several crops, including tomato, rice and barley (Tanksley *et al.,* 1996; Fulton *et al*., 1997, 2000; Bernacchi *et al.,* 1998; Xiao *et al.,* 1996, 1998; Moncada *et al*., 2001; Pillen, Zacharias and Léon, 2003, von Korff *et al*., 2008). Once favourable QTL alleles from an exotic donor are identified, one or two additional backcrossing and selfing generations are needed to derive QTL-bearing near-isogenic lines (QTL-NILs). These carry recurrent parent alleles throughout their genome except for the specific target QTL (Tanksley and Nelson, 1996). QTL-NILs can be used to verify observed QTL effects as well as commercial lines improved for one or more quantitative traits compared with the original recurrent elite line.

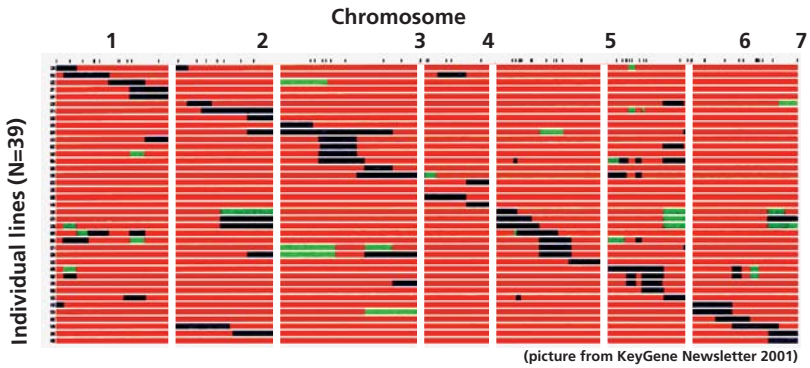
In contrast to the AB-QTL method, Eshed and Zamir (1994, 1995) suggested the approach of establishing a population of NILs such that the donor chromosome segments are evenly distributed over the whole recipient genome. Ideally, the total genome of the exotic donor is comprised in the established set of NILs (Figure 5.9). This NIL population, termed an introgression library, consists of a set of lines, each carrying a single marker-defined donor chromosome segment introgressed from an agriculturally unadapted source into the background of an elite variety (Zamir, 2001).

The procedure of establishing an introgression library implies systematic transfer of donor chromosome segments from a PGR (donor) into an elite line (recurrent parent) by marker-aided backcrossing. Additional self-pollination and marker-based selection lead to NILs homozygous at donor chromosome segments. Such NILs differ from the elite line by only a small, defined chromosomal segment, and phenotypic differences between a line in the library and the nearly isogenic elite line are associated with the single donor chromosome segment (Šimić *et al.*, 2003).

Both introgression library and AB-QTL approaches provide a valuable opportunity to extract quantitative trait alleles for modern crop varieties from exotic PGR. Their main advantage is that the exotic genome is

FIGURE 5.9
Graphical illustration of an introgression library

Using graphical genotypes to illustrate an almost ideal introgression library that covers most of the donor genome



Homozygous region of the donor
Heterozygous regions
Homozygous regions of the recurrent parent

introgressed into the elite line only as small, well defined donor chromosome segments. This reduces unfavourable effects that often impede the use of PGR in practical breeding programmes.

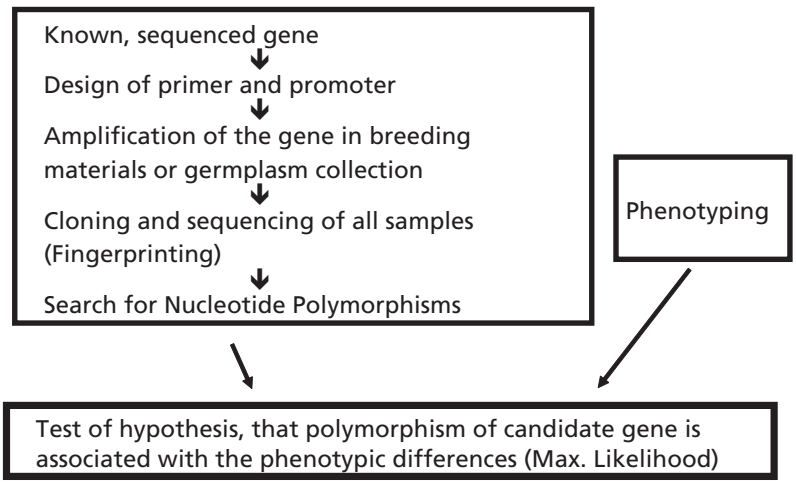
5.7.4 Association studies and direct allele selection

Increased insight into the molecular organization and sequence of plant genomes has led to new methods to mine directly the allelic diversity of PGR. The aim of such studies is to associate sequence polymorphisms within genes or across genomes with phenotypic variants to detect superior alleles affecting agronomically important traits. Such valuable alleles detected within germplasm collections can subsequently be transferred to elite breeding materials via marker-assisted backcrossing using allele-specific markers (direct allele selection; Sorrells and Wilson, 1997) or marker-assisted recurrent selection (D. Hoisington, pers. comm.). The major advantages of association studies over classical QTL mapping experiments is that no segregating population has to be established from two inbred lines, and that the results are not limited to the specific mapping population but can cover the full allelic variation available in natural or breeding populations or gene bank accessions (Jannink, Bink and Jansen, 2001; Jannink and Walsh, 2002).

Associations between DNA sequence polymorphisms and phenotypic trait variation can occur either when the polymorphisms are directly responsible for the functional differences between the alleles of the respective genes, or when the analysed polymorphisms are in linkage disequilibrium (LD) with the functional alleles. LD is defined as a non-random association of alleles at different loci within a population (Falconer and Mackay, 1996).

The basic idea of association mapping can be investigated using two strategies.

FIGURE 5.10
Steps in an association study using the candidate-gene approach



One approach is first to identify candidate genes (i.e. from available databases or gene expression studies) and to re-sequence those candidate genes in plants derived from diverse germplasm accessions (Figure 5.10). The maize gene *dwarf8*, a candidate gene for flowering time and plant height, was used by Thornsberry *et al.* (2001) in a first association study with a crop species. They sequenced *dwarf8* in a representative set of 92 inbred lines and found polymorphisms within the gene to be strongly associated with flowering time. This group of researchers also developed a software suite, TASSEL, (http://www.maizegenetics.net/bioinformatics/index.htm) for analysing LD and for performing association mapping in populations of inbred lines.

A second approach is to analyse a set of randomly chosen molecular markers, evenly distributed across the genome. If such markers are in LD with the genes controlling the trait variation, one will also detect a significant association. The practicability of this approach strongly depends on the level and structure of LD. Low levels of LD would be favourable for high resolution fine mapping within candidate genes, but limit the feasibility of genome-wide association studies. A first attempt to use the genome-wide approach in plants was reported for *Beta vulgaris* subsp. *maritima* using 440 AFLP markers in 106 individual plants from four natural populations (Hansen *et al.*, 2001). Two markers were detected showing significant association with the bolting gene, which is responsible for the vernalization requirement.

Population structure in germplasm collections, which may be unknown to the researcher, can cause spurious associations. Statistical methods were developed by Pritchard, Stephens and Donnelly (2000) and Falush, Stephens and Pritchard (2003) to detect such population structures using a few molecular markers evenly spread across the genome. Removing the effects of population structure increases the power of the association study to detect useful markers.

5.8 THE USE OF GENETIC RESOURCES IN PARTICIPATORY PLANT BREEDING

Genetic resources can be used in a number of ways in participatory plant breeding programmes.

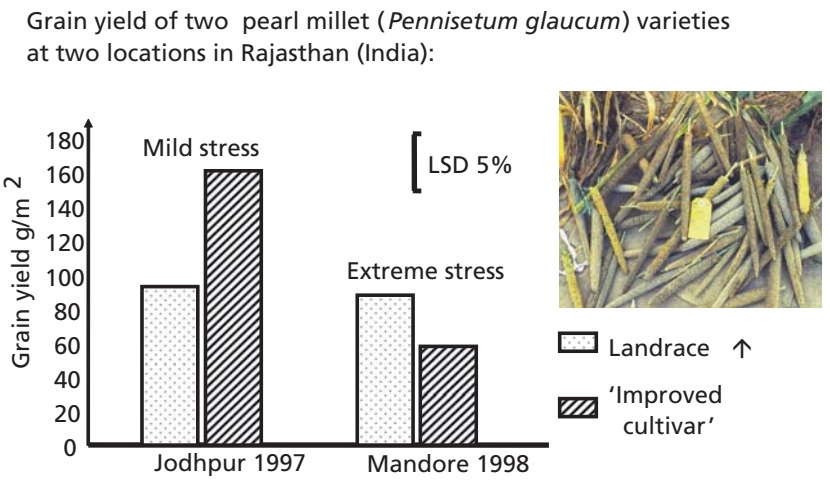
Participatory improvement of diversified populations and potential contribution to in situ *conservation of PGR*

Farmer-participatory improvement of diversified populations combines *in situ* conservation with genetic improvement of PGR to meet farmer's diverse needs as well as the challenges of adaptation to site-specific conditions, climatic variability and climate change. In a first step, farmers may evaluate a range of diverse varieties or germplasm accessions of the target crop and chose accessions that carry traits of interest to them. The diversified base population will then be built through crossing and recombining the farmer-selected materials. Representative seed lots of targeted base populations will be distributed to farmers in contrasting sites with specific selection pressures of a target region (see Figure 5.8 above). Natural and recurrent selection by farmers and breeders will act on the distributed material and lead to the development of new subpopulations that can be excellent sources of variation for specific adaptation and farmer-preferred traits, as well as new trait combinations (via recombination) not previously available. Such a dynamic gene pool approach provides the best opportunity to "offer a wide diversity of material to the wide diversity of farmers" for effective participatory plant breeding (Weltzien *et al.*, 2000).

Use of landraces as genetic resources for specific adaptation to stress environments, climate variability and climate change, and to better serve farmer's and end-user's diverse needs

Breeding for wide adaptation has been found to be inappropriate for extreme stress environments, because of cross-over genotype × environment interactions appearing at low yield levels (e.g. Simmonds, 1991;

FIGURE 5.11
Example of the utility of local landraces as source of adaptation to extreme growing conditions



Ceccarelli *et al.*, 2001; vom Brocke *et al.*, 2002a, b). Cross-over genotype × environment interactions represent the situation where newly bred 'widely adapted' cultivars are inferior to local, indigenous varieties under extreme environmental conditions. An example is given in Figure 5.11. Such interactions may be considered as a hindrance to crop improvement in a target region, but they also offer new opportunities, e.g. selecting and using genotypes that show positive interaction with the location and its prevailing environmental conditions (exploitation of specific adaptation), or genotypes characterized by low frequency of crop failure (Annicchiarico, 2002).

Landraces grown in extreme areas, such as semi-arid to arid regions in Asia and Africa, can represent important PGR in breeding for specific adaptation (Hawtin, Iwanaga and Hodgkin, 1997). They can be donors for individual monogenic traits; sources of new quantitative variation for specific adaptation to stress conditions; and breeding population or crossing partner in the development of improved, locally adapted cultivars for the same or other marginal areas. Strategies for the development of locally adapted germplasm include (Ceccarelli *et al.*, 2001; Witcombe, 2001; Ceccarelli and Grando, 2007):

- decentralization of the breeding process from the international to the national level, and from stations to farmers' fields;
- crossing of elite materials with locally adapted, farmer-preferred cultivars;
- development of different breeding populations for different regions;
- distribution of segregating materials to national programmes; and
- farmer-participatory selection, to increase final acceptance of the improved cultivars.

5.9 OUTLOOK

Numerous methods are available for the use of PGR in crop improvement. The choice mainly depends on the crop, the trait(s) of interest, availability of molecular markers, the chosen time frame, and the finances available. A combination of advanced, molecular techniques with classical and farmer-participatory breeding methods will most probably achieve the desired impact. In order to enhance the utilization of PGR in crop improvement, the Global Plan of Action (FAO, 1996b) proposed a number of measures, among them expanded creation, characterization and evaluation of core collections; increased genetic enhancement and base-broadening efforts; development and commercialization of underutilized species; development of new markets for local varieties and 'diversity-rich' products and concomitant efficient seed production and distribution; comprehensive information systems for PGR; and promoting public awareness of the value of PGR for food and agriculture.

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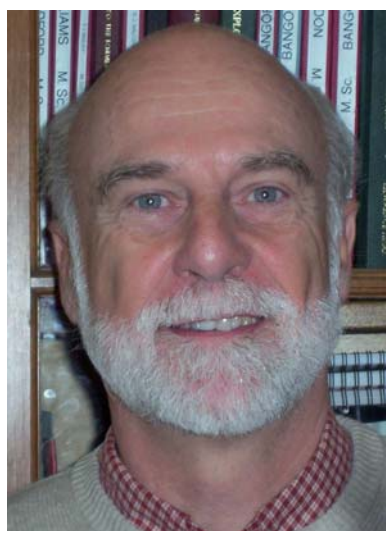
CHAPTER 6

Methodologies for generating variability Part 2: Selection of parents and crossing strategies

John R. Witcombe and Daljit S. Virk



6.1 INTRODUCTION

More client-oriented approaches to plant breeding actively involve farmers in either consultative and collaborative roles, or both, in early generations in the breeding process (see Witcombe *et al.,* 2005; IPGRI, 1996). Little attention has been paid as to how the methods might differ from classical breeding in the number of crosses that are made and hence the strategy for the selection of parents.

In conventional breeding of inbred crops on research stations, breeders have the capacity to deal with the progeny of hundreds of crosses each season. Even with fairly limited resources, they can test many hundreds, or even thousands, of F_4 or F_5 lines in a nursery that has no, or few, replicates. In cross-pollinating crops, dozens of composites can be handled and trials can be conducted on hundreds of progenies (for population improvement) or inbred lines (for hybrid breeding).

Farmers can only grow variable material in their own fields if there are many fewer crosses, entries and plots than in classical breeding, because, without help from scientists, individual farmers cannot be expected to grow trials of hundreds of entries. However, in a participatory breeding programme it is inexpensive for a farmer to grow a very large population of any entry when it replaces the usual cultivar. The cost to the farmer of replacing his or her usual variety is only any decrease in the value of the crop, not the total cost of growing the crop. Indeed, when the segregating population is superior to the customary variety it provides a benefit, whereas, in classical breeding, the full cost of the area under an increased population size is borne by the breeding programme. Hence, in a participatory programme it is cost effective to have a farmer grow large bulk populations, and this can easily be replicated by collaboration with several farmers.

6.2 NUMBER OF CROSSES

How many crosses are used in a breeding programme has crucial impacts on success and efficiency. However, the outcomes of theoretical calculations to determine the optimum number of crosses vary greatly with the assumptions that are made on how well the breeder can predict the value of crosses. If the breeder can predict the best cross with certainty, then only one cross is needed, but, assuming the breeder has no power of prediction at all, very many are required. These are extreme assumptions but neither experimental data nor theories exist that determine where the balance lies between the two. Hence, most breeders have inclined, undoubtedly with much success, towards what seems to be a more risk adverse strategy of the latter extreme, with many programmes having hundreds of crosses per year (Witcombe and Virk, 2001). Can efficiency be improved by moving towards the former, rarely-tested, extreme of using only a few crosses?

This question cannot be answered from experimental approaches on the optimization of cross number and population size. We have found no such reports in the literature, presumably because the required experiments are too large and expensive. An ideal comparison is clearly very difficult: it would compare the results of two parallel breeding programmes conducted over many years that use the same total number of plants (K), identical selection methods and environments, but contrasting values for m (number of crosses) and n (population size).

Although data and practical theory are lacking on how many crosses to use, the

theory is clear that the optimum population size of any cross has to be large if desirable transgressive segregants are to be obtained for traits that involve several loci (Allard, 1999). However, most breeding programmes use much smaller population sizes than theory dictates to accommodate the many crosses that are made.

6.2.1 A re-examination of the theory on the optimum cross number

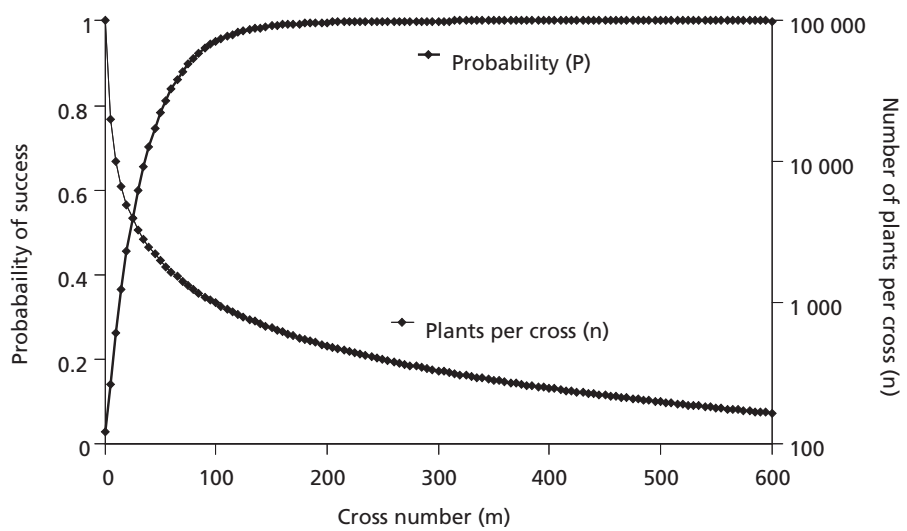
In theoretical determinations of the optimal number of crosses (*m*) and population size (*n*) per cross, given a limit of *K* plants, two approaches have been used: either (i) minimizing the risk of excluding superior genotypes, or (ii) maximizing the response to selection.

Using the first approach, Yonezawa and Yamagata (1978), Wricke and Weber (1986) and Hüehn (1996) suggested increasing the number of crosses rather than increasing the population size of each cross in order to minimize the risk of missing the favourable plant. They found that each of the *m* crosses should be represented by only one F_2 plant ($n = 1$) to give the lowest risk of failure. However, this did not consider all of the possible scenarios. The probability of success can increase to 1 long before *n* falls to 1. This happens when:

- the probability of a cross succeeding (*P*1) is high, but still much lower than what we have achieved in our few-cross breeding programmes;
- the probability of a plant succeeding (*P*2) is also reasonably high; and
- *K* is large (Figure 6.1).

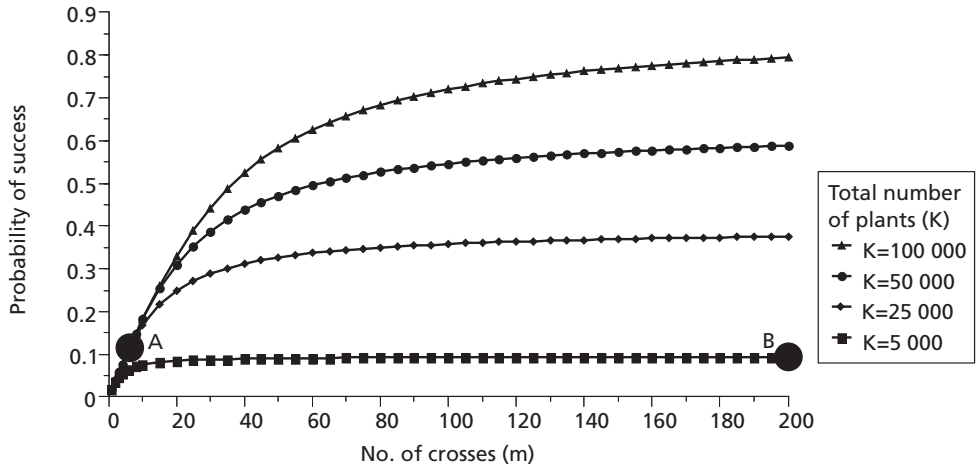
In the second approach, the magnitude of the response to selection among and within crosses is considered (Baker, 1984; Wricke and Weber, 1986; Hüehn, 1996;

FIGURE 6.1
The probability of success (left hand y axis) with a varying number of plants per cross (right hand y axis log scale) for 1 to 600 crosses



*P*1 (probability of cross succeeding) = 0.03, *P*2 (probability of a plant succeeding) = 0.005 and *K* (total size of population) = 100 000 in all cases.

FIGURE 6.2
Effect on the probability of success of varying the number of crosses (m) with four constant total population sizes (K)



*P*1 (probability of cross succeeding) = 0.02, and *P*2 (probability of a plant succeeding) = 0.001 in all cases. Point A is at 6 crosses with *K* = 100 000 and point B is at 200 crosses with k = 5000.

Bernardo 2003) and fewer crosses where *n* is always >1 maximizes the chance of success. For example, Baker (1984) concluded that for a total of 2 000 families the maximum response is found with 50 to 100 crosses having 40 to 20 families. Therefore, Bernardo (2003) concluded that the two approaches give conflicting results. However, we have found that by examining the response to increasing the cross number (*m*) the conflict is not as great as it might first appear. If the values of *K* are high, then adding more crosses is not very effective in increasing the probability of success, and a fewer-cross strategy with a larger population size would be more cost effective (see below for considerations of cost). Using the model of Yonezawa and Yamagata (1978), the rate of increase in the probability of success by adding more crosses declines dramatically after about 50 to 100 when values of *K* are ≤50 000 and values of *P*1 and *P*2 are not extreme (Figure 6.2). Moreover, when values of *K* are greater than this, e.g. 100 000, then adding crosses improves the probability of success very little after about 15 crosses are made (Figure 6.2).

Even though the model of Yonezawa and Yamagata (1978) has been used to argue the case for more crosses, the argument for increasing *K* is just as powerful. A few crosses where *K* is large have a much lower risk over many crosses when *K* is small. For example, 6 crosses (point A in Figure 6.2) where *K* = 100 000 are less risky than 200 crosses (point B in Figure 6.2) where *K* = 5 000.

The relative costs of making crosses and growing plants are ignored in the model of Yonezawa and Yamagata (1978). Hence, with one cross or many crosses *K* remains the same, even though it is easier and cheaper to make one cross and grow 10 000 plants from it than make 10 000 crosses and only grow one plant from each. This applies

to all other values, such as 200 crosses with 50 plants each compared with 100 crosses with 100 plants each. Not only is there the additional cost of making more crosses, but there is an additional cost in record keeping, planting and labelling the more crosses there are within any given *K*.

In all of these models we have not considered the ability of breeders to choose superior crosses rather than making random ones. If the declining probability of success of each cross is considered—the first choice of a breeder should be better and more carefully considered than the hundredth—then the optimum number of crosses falls. This is simply a quantitative and realistic extension of the argument that if the best cross is known then only one needs to be made. The optimum number of crosses also becomes smaller as more resources are spent on evaluating parents and more time is spent on choosing crosses.

6.3 CHOICE OF PARENTS

6.3.1 Selection of parents

Little consensus exists among plant breeders on how best to choose parents for crosses that will produce high yielding progenies and it remains a debated issue (Qualset, 1979; Baker, 1984). The strategies for selection of parents for desired progeny performance fall into two categories: methods based on parental performance *per se*, or methods that assess the value of parents estimated from progeny performance. The first category includes selection based on: mid-parental values; divergence coefficients among parents (Murphy, Cox and Rodgers, 1986); character complementation or the geometric approach (Grafius, 1964, 1965; Lupton, 1965); and multivariate analysis and parental distances (Bhatt, 1970; Pederson, 1981). These methods have the advantage that they use data from a single generation. However, the efficacy of these methods can only be evaluated by progeny tests.

In the second category of methods, parents are evaluated on the basis of the performance of their progeny. Such tests require time as at least two generations of plants need to be grown and evaluated to determine means and variances. The evaluation can be of combinations of F_1, F_2 and later generations (Allard, 1956; Busch, Hanke and Frohberg, 1974; Cox and Frey, 1984) or the evaluation of progeny produced from mating designs such as diallel and line × tester (Lupton, 1965). The relationship between predicted and actual progeny performance provides empirical evidence of the value of selecting parents by these methods, but the results of such experiments provide no consensus.

Other authors have concluded that gathering experimental data to estimate the value of possible crosses using progeny tests demands so many resources that it is unwarranted (Wricke and Weber, 1986; Lupton, 1965). We would agree with this as participatory methods provide simpler and effective methods of choosing parents by using genotypic performance *per se* as a prediction of parental value (Baenziger and Peterson, 1992). This is done without using the formal quantitative analysis described above for the first category of methods. Much information is already available for genotypes that have already been adopted by farmers. If a breeder's knowledge of such genotypes can allow the prediction of the more useful cross combinations, then only a few would need to be made, each with a higher probability of success. We have demonstrated in practical, participatory breeding programmes that this is the case in rice (Joshi *et al*., 2007; Virk *et al*., 2003) and that the equivalent in maize to a few

crosses—a single composite population—is also effective (Witcombe, Joshi and Goyal, 2003; Virk *et al*., 2005).

The breeder can predict that a cross is more likely to give rise to desirable segregants when the parents have complementary attributes suitable for the target environment. It is advantageous if they are also unrelated, to increase the extent of possible transgressive segregation. A few-cross, participatory strategy emphasizes the role of the plant breeder in the evaluation of introduced and collected germplasm as potential parents and the collective skills of farmers and breeders in selection, rather than emphasizing the skill of the breeder in selecting superior genotypes from within many crosses.

In participatory, i.e. decentralized, breeding at least one of the parents should be adapted to the target environment and have traits that farmers like. Participatory varietal selection (PVS) efficiently identifies locally adapted parents: a range of germplasm can be evaluated by farmers in their own fields that can include local landraces, recommended cultivars and introduced varieties. A variety selected by PVS is an ideal parent since it has local adaptation and traits that farmers prefer. Witcombe *et al.* (1996) suggested several types of crosses following PVS: a variety selected by farmers in the PVS trials is crossed with either a local landrace, another variety selected by participatory methods, or an exotic variety. This allows crosses to be made both between adapted × adapted parents or between adapted × unadapted parents. Of these options, an adapted variety identified by PVS crossed with an unadapted exotic variety will often have the greatest genetic dissimilarity. When breeding for marginal environments, the exotic variety can have adaptation to more favourable environments and hence be selected for its high yield potential and multiple disease and pest resistance. Client-oriented, participatory methods then benefit from classical breeding for adaptation to favourable environments.

Another participatory method conceptually closely related to PVS is to exploit current varietal adoption. D.N. Duvick (pers. comm.) has pointed out how farmers do a tremendous amount of selection for maize breeders in the United States of America, because the inbred-line parents of the most successful cultivars are used as parents in breeding programmes. This is participatory research—those cultivars have been grown over thousands of locations for several years providing a multilocational testing system far beyond the capacity of a formal breeding programme. In our rice breeding programmes in Nepal, widely adopted varieties such as CH 45 and Sabitri have been used as parents. Although extent of current adoption is clearly a useful criteria for parental selection, in many marginal areas PVS will sometimes quickly and cheaply identify varieties superior to those that farmers are currently growing, e.g. Joshi and Witcombe (1996).

Landraces may sometimes offer specific characteristics that are preferred by farmers. However, in our breeding programmes in rice, using landraces as parents has been less productive than using high-yielding modern varieties. Perhaps this is unsurprising, since improved varieties often have higher yields, and are more disease resistant than landraces. It makes no sense to use landraces just because they are landraces (Wood and Lenné, 1997), but rather to use them only when identified as having superior attributes. The argument that a landrace is 'locally adapted' and has post-harvest qualities that farmers appreciate is

insufficient when the best PVS variety also has these traits.

The strategy for choosing parents may differ little across target environments and the scale of the breeding programme. More favourable environments are highly diverse and participatory approaches are also needed for them (Witcombe, 1999). In breeding for favoured mega-environments, the same crosses are used to cover several or many countries, but this process is better decentralized by matching crosses to target countries (Ceccarelli *et al.*, 1994).

6.4 EVIDENCE AND CONCLUSIONS

We have used a few-cross approach in our client-oriented breeding (COB) programmes (Witcombe *et al.*, 2005). Using few crosses was effective; we made only three crosses in our breeding programme for rice targeted at Nepal and India by 1998, two of which were clearly successful, compared with a success rate of <1 percent in classical breeding programmes (Witcombe and Virk, 2001). The first cross we made, between the tall upland rice variety Kalinga III and the dwarf-statured lowland IR64, produced successful varieties, including three that had been released by 2008: two in India (Virk *et al.*, 2003) and one in Nepal (Gyawali *et al.*, 2006). The third cross we made, Radha 32/Kalinga III, produced Judi 582 and Judi 572 that have been adopted in Bangladesh (Joshi *et al.*, 2007). The second cross we made, Kalinga III/IR36, also produced high yielding lines, but these were not promoted as they were inferior in grain quality to those from the IR64 cross.

A fewer-cross strategy greatly simplifies the breeding scheme and saves resources. Some resources were re-allocated to increase the probability of obtaining desirable segregants (and reduce the risk from using only a few crosses) by using a much larger F_2 population from which several generations of large, early-generation, bulk populations were derived before lines were produced (Witcombe and Virk, 2001). Thus we avoided using resources on selection in the early generations, when it is less efficient for low-heritability traits compared with selection in later ones (Fahim *et al.*, 1988). Instead, we concentrated resources on selecting in later generations when higher between-line genetic variance increased efficiency (Kearsey and Pooni, 1996). Mass selection in the advanced bulk populations produced rice varieties as uniform as those from line selection (Virk, Steele and Witcombe, 2007).

There is further evidence that the few-cross approach is effective. Another rice breeding programme in Nepal funded by the International Plant Genetic Resources Institute (IPGRI, now Bioversity International) has also relied on only a few crosses. One parent was always a local landrace because landrace utilization was an objective of the programme. Even with this constraint on the choice of parents, of only 8 crosses, 4 have resulted in varieties that are in the release or pre-release stage (Gyawali, unpublished). At the Africa Rice Center (WARDA; formerly the West Africa Rice Development Association), a 'wide-cross' breeding programme between *Oryza sativa* and *O. glaberrima* placed considerable effort on choosing the parents of the crosses (Jones *et al.,* 1997). Only eight parents of *glaberrima* and five of *sativa* were chosen on the basis of their best combination of traits, and only seven of the crosses set seed. All of the seven 'New Rice for Africa' (NERICA®) varieties that were released in 2000 (WARDA, 2006) were from just one of these crosses, a success rate of 14 percent. As was the case for our crosses, this is a considerable improvement over normal success

rates and our experience suggests that this was due to the great attention paid to choosing parents, necessitated by the high cost of making these wide crosses. In maize, the parallel of a few-cross approach is to make only a single composite population, and we tested this in western and eastern India. Two populations were made, one for each region, and both have produced a released variety (Witcombe, Joshi and Goyal, 2003; Virk *et al.*, 2005).

What if all breeders used only a few crosses? This would restrict the amount of germplasm used in crosses but not restrict the amount used in successful crosses. In conventional programmes, although many crosses are made, most produce neither released varieties nor progeny that could be used in crosses to eventually produce a released variety. However, the exceptions are valuable: for example, IR64 has an extremely complex parentage with 20 original farmer varieties from eight countries as parents (IRRI, 1985). Clearly, not all of them would have previously been released varieties or parents of released varieties. To deliberately broaden the genetic base of crops, more crosses have to be made. This is particularly so when it involves parents about which little is known or, as is the case for little-grown landraces or wild relatives, when performance *per se* gives poor indications that the parents are valuable. Using few crosses is certainly suitable for breeding that is entirely targeted at rapidly producing varieties for client farmers in national programmes that have limited resources. The few crosses allow better market orientation and increase efficiency.

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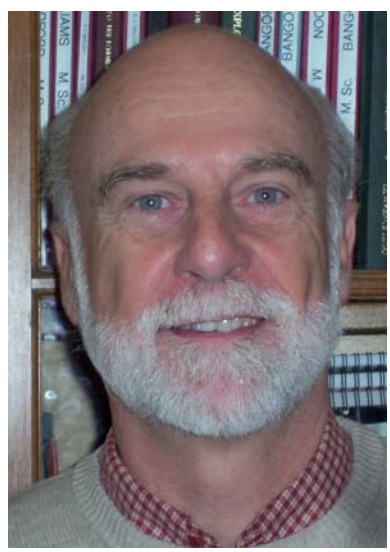
CHAPTER 7

Methodologies for generating variability Part 3: The development of base populations and their improvement by recurrent selection

John R. Witcombe



7.1 INTRODUCTION

This chapter presents a detailed account of the methodology of population improvement in open-pollinated crops using maize (*Zea mays* L.) and pearl millet (*Pennisetum glaucum* (L.) R. Br.) as examples. These are examples of highly cross-pollinated crops, for which population improvement breeding methods are very suitable. Appreciable gains for grain yield have been realized by recurrent selection in maize (Hallauer and Miranda, 1988) and in pearl millet (Govil, Pokhriyal and Murty, 1982, 1986; Kapoor *et al*., 1983; Khadr, 1977).

Population improvement methods can be used both in the breeding of hybrids and in the breeding of open-pollinated varieties (OPVs) or the two can be combined. Hybrids tend to be higher yielding, but OPVs are easier to breed, their seed production is cheaper and quicker, their disease resistance is more stable over seasons and locations, and low-resource farmers can cultivate the crop from farm-saved seed without paying a large yield penalty. In this chapter, neither hybrid breeding nor combinations of hybrid breeding and open-pollinated variety breeding are considered.

Useful farmer participatory techniques that improve the client orientation of the programme and its effectiveness are considered. These techniques increase the possibilities of successful use of the new varieties by the client farmers.

7.2 FORMATION OF BASE POPULATIONS

7.2.1 How many parents

Few or many parents can be intercrossed to form base populations for recurrent selection. The number will depend on the genetic diversity among them and the balance desired between high initial yield (to increase short-term gains) and high genetic variance (to increase the potential for long-term genetic advancement). Because of the need to balance short- and long-term gains, there is no generally applicable rule for determining the optimum number of parents. An additional factor is the degree of inbreeding in the parents; to avoid inbreeding depression, more inbred lines are needed than if open-pollinated cultivars are parents.

7.2.2 Choosing parents

Methods that actively involve the target clientele can quickly identify the traits needed in parents. This is done poorly in many breeding programmes, as shown by farmers continuing to grow landraces or obsolete cultivars while officially-released cultivars lack the traits demanded by them. Surveys of farmers are needed to elicit information on what is needed. In the public sector, such surveys are often referred to as participatory rural appraisals (PRA) (Chambers, 1997) and are equivalent to the market research approach of the private sector (Sumberg and Reece, 2004). For a breeding programme, well-applied PRA techniques or customer profiling result in better client orientation and make possible efficient goal setting (e.g. Weltzien, Whitaker and Anders, 1996). In pearl millet, for example, Kelley *et al*. (1996) identified the importance of traits such as straw production and quality. At the end of this process a product specification can be made where the traits of the desired variety are known for all of the major adaptive, yield and quality traits (Witcombe *et al*., 2005).

The above is a consultative process, but the active collaboration of farmers in participatory varietal selection (PVS) helps greatly when parents are selected for their performance *per se*. Techniques that delay the creation of the base population by, for example, running yield trials or conducting genetic analyses on candidate parents (e.g.

Gardner and Eberhart, 1966) are usually not warranted. This is particularly true for client-oriented approaches where often the breeding is targeted at farmers who have had limited benefits from modern plant breeding. The base population then needs to be developed from a viewpoint of speed and simplicity, because the task is not the difficult one of improving upon a recent modern variety but to improve upon a landrace or an obsolete variety.

The biggest gains will be made when the population has a high initial yield and a high genetic variance. For decentralized breeding, we have found that the recombination of locally adapted varieties with high-yielding exotic varieties can produce this desirable combination. High genetic variance can be achieved by crossing unrelated germplasm, such as white- and yellow-endosperm material (Witcombe, Joshi and Goyal, 2003; Virk *et al*., 2005). Tiwari (2001) tried several types of crosses, and the one that involved both local and improved germplasm and white- and yellow-endosperm types was the most successful.

In client-oriented breeding programmes that are decentralized to a given target region, a high proportion of the base population parents should be adapted to it and have traits that local farmers like (Witcombe *et al*., 1996). To increase diversity, less-well-adapted parents should also be included for their complementary attributes in order to produce desirable segregants. When breeding for marginal environments, these complementary parents can be adapted to more favourable environments and are chosen for high yield potential and superior disease and pest resistance. They are likely to be unrelated to the locally adapted parents, thereby increasing the genetic variance of the population. In pearl millet, the value of such an approach of crossing locally adapted landraces with complementary, high yielding parents (modern varieties) was demonstrated (vom Brocke *et al*., 2002, 2003). The introgression of modern varieties into landraces increased genetic diversity, led to broader adaptation than either landraces or modern varieties alone, and under high rainfall conditions still yielded as much as modern varieties.

In maize, in western India (Witcombe, Joshi and Goyal, 2003) and in eastern India (Virk *et al*., 2005) the traits that farmers wanted in new maize varieties (white grain (endosperm), with early maturity and with tolerance to the most common abiotic constraints of drought encountered in these regions) were determined from interviews and from the results of participatory trials. More detailed requirements emerged during the course of these programmes, such as a need for high cob-placement to avoid damage by jackals. In Africa, Bänziger and Cooper (2001) have targeted the two major constraints identified by farmers, namely drought and the ability to yield well even under low nitrogen conditions. In pearl millet, a survey of farmers (ICRISAT, 1987) in Maharashtra showed the importance that farmers placed on large individual grain size and early maturity. A breeding programme was based on the creation of a composite with bold grains and earliness (the Bold Seeded Early Composite). This produced highly acceptable cultivars for farmers in both India and several African countries (ICRISAT, 1997).

Participatory varietal selection is a very efficient way of identifying locally adapted parents with traits that meet specific client needs. A closely related method, because it also relies on farmer acceptance of varieties, is to exploit knowledge of current varietal adoption. Duvick (2002) points out that farmers do a tremendous amount of selection

for maize breeders in the United States of America, because the inbred-line parents of the most successful cultivars are used as parents in breeding programmes. However, although current adoption is clearly a helpful criterion for parental selection, in more marginal agricultural areas it may be less useful. There may be considerably better varieties than those that farmers are currently growing and these can be quickly and cheaply identified by PVS (e.g. Joshi and Witcombe, 1996).

7.2.3 Population size

Population size has to be large to provide a reasonable probability of finding rare or infrequent desirable transgressive segregants. In cross-pollinated crops, small population sizes cause significant inbreeding depression, so population sizes need to be sufficiently large to avoid this; an effective population size of more than 500 plants in each generation is sufficiently large to avoid significant inbreeding depression (this is calculated from a standard formula, e.g. Falconer, 1981; see also Chapter 2 in this volume). The resources available also dictate the size of each population. The more base populations that are created and improved, the fewer the resources that can be devoted to each one. In the breeding of open-pollinated crops, creating and improving even a single population still requires many resources. The strategy used in our client-oriented breeding programmes has therefore been to minimize the use of resources by improving a single population for each group of target clients (Witcombe, Joshi and Goyal, 2003; Virk *et al*., 2005). Not only does this reduce the resources required, but it more carefully focuses the base population to an identified target group of clients. It has proven to be a successful strategy.

7.2.4 Making the initial cycle (C_0) bulk

General considerations

Adequate genetic recombination between the parents of a composite will produce a diverse range of recombinants for the first cycle of recurrent selection. How early this occurs will depend on how inbred the parents are. When the parents are inbred, the third generation of random mating is equivalent to the F_2 in an inbreeding crop in that it is the first in which transgressive segregation can occur. In this case a third generation of random mating is needed before selection should commence. Transgressive segregants occur earlier if the parents are heterozygous, open-pollinated varieties. In this case, mass selection after a single generation of random mating can be expected to result in a genetic advance, but possibly at the expense of an early reduction in genetic diversity that reduces the potential for long-term gains. A third generation of random mating remains desirable before any progeny testing is started.

Using maternal ancestry to aid recombination

In crops where the occurrence of natural selfing is low, the simplest method of recombination is to allow random mating in a bulk grown from equal amounts of parental seed. However, the maintenance of some form of population structure is desirable as it allows a visual estimate to be made of the extent of recombination. When parental numbers are not too high, this can be done by maintaining sub-bulks derived from the individual parents of the base population and growing them in an isolated plot (Figure 7.1). To aid randomness of recombination a pollinator bulk is used that is made from aliquots of seed of the original parents. The pollinator bulk is preferably also planted on the borders of the isolated

FIGURE 7.1
Planting layout design for hills in maize. Detasselled plants in grey

6	3	2	5	**8**	2	1	7	1	5	**1**	2	4	2	8	5	7	8	6	3
4	9	4	6	4	8	9	8	9	3	**7**	**1**	8	**1**	6	1	6	1	4	9
7	8	2	9	7	5	4	5	6	8	4	**7**	**1**	**3**	5	8	5	**2**	**7**	8
2	**7**	1	5	1	**3**	**7**	**3**	4	5	**6**	2	**3**	8	7	3	1	**7**	**2**	**7**
5	4	6	**2**	**9**	**7**	**3**	1	8	**6**	3	9	7	9	2	9	4	3	5	4
1	**9**	**2**	8	**2**	**9**	8	9	5	7	**6**	4	8	5	**3**	6	2	3	1	9
9	**2**	3	9	5	6	3	2	1	**2**	**9**	3	**2**	**3**	8	4	**1**	7	9	2
3	**8**	**2**	7	**3**	**4**	9	5	**6**	**9**	**2**	1	5	**2**	1	7	8	**1**	3	**8**
8	5	4	**3**	**4**	8	**4**	**6**	2	1	7	3	4	7	9	2	9	5	**8**	5

plot. When, for example, 50 percent of the area is planted with the pollinator bulk, the entries and the bulk are planted in alternate ridges and beds so that every entry is surrounded by the pollinator bulk. In the second and subsequent random mating generations, the pollinator is either recreated by bulking aliquots of seed taken from the open-pollinated entries, or is advanced through the generations by harvesting its open-pollinated seed (Figure 7.1). Random mating is repeated until the entries lose all or most of their identity relative to each other and the pollinator bulk. In maize, unlike in pearl millet, the entry rows and the pollinator bulk can be made to cross by detasselling the entry rows. In this case, the pollinator bulk is best made up afresh each time from aliquots of the female entries.

If necessary, only the entry rows can be planted, either to reduce the land requirement or because there is insufficient seed to sow both the entry rows and the pollinator bulk. The extent of recombination is then assessed by the between-entry phenotypic differences.

When recombination appears complete, equal amounts of seed are taken from the entry rows to make the C_0 bulk of the composite. A portion of the C_0 bulk should be retained for use as the base population in trials for evaluating the progress made by selection.

Using forced crossing in the first generation of random mating

When there are few parents, it is much more efficient to employ diallel crossing in the first generation of random mating. This greatly increases randomness of mating by avoiding the high proportion of sibbing within the parental entries that occurs under natural random mating. It also avoids the need for an isolated plot. When there are too many parents to make a complete diallel, a half diallel can be used, or they can be crossed in, for example, a partial diallel using systematically selected crosses, or a randomly made partial diallel (designs for such random crossings are discussed below). If there are not too many crosses in the diallel, then progress of extent of recombination can be assessed, in the second and subsequent random matings, by planting the entry rows according to the maternal parents of the cross that were made in the first generation.

Hill designs in maize

In maize, the prevention of selfing is simply done by detasselling plants. This can lead to very effective methods of increasing

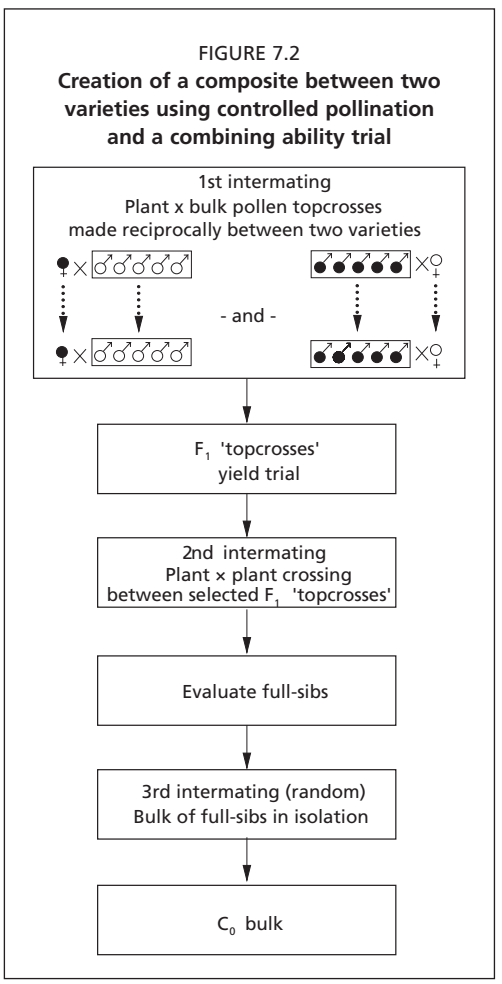
recombination. In the breeding of maize using client-oriented methods only a single composite was made per target environment so resources allowed quite sophisticated methods to be used in the creation of the base population. Witcombe, Joshi and Goyal (2003) described the making of a base population based on crossing three varieties with white endosperms and three with yellow endosperms. All nine possible white by yellow crosses between the varieties were made by hand pollination in a reciprocal fashion. In this generation, selfing was avoided by selecting only grains of the colour of the pollen parent; endosperm colour is a highly heritable trait and xenia makes the pollen genotype apparent in the seed in the maize ear. In subsequent generations, hills were planted of the nine crosses in a pseudo-random design: the hills were randomized but plants derived from the same cross were not allowed to occur in adjacent hills in either the horizontal or the vertical rows. Where adjacent hills in a diagonal row were from the same cross the plants were detasselled and further plants were randomly selected for detasselling to bring the proportion of detasselled plants to 50 percent. An example of part of the planting design is shown in Figure 7.1, with detasselled plants in bold, before the addition of the randomly selected plants. Hills were individually harvested and labelled with the cross number to repeat the design in the following generations.

There are simpler ways of attempting to maximize recombination, such as using the row-planting model shown in Figure 7.1. However, given that with client-oriented methods only one base population is used, more resource-consuming methods are feasible and reduce the perceived risk of relying on a single base population.

Recombination with progeny testing

In the most complex methods, progeny are tested during the random mating generations. For example, when only two varieties or composites are merged to form a new composite, then the combining ability of individual plants of one entry can be assessed by using the other entry as the tester. The topcross hybrids produced by individual plant × bulk pollen crosses are assessed in a yield trial, and the selected topcross hybrids are themselves used as parents for the second generation of random mating (Figure 7.2). The topcross test is preferable to making full-sibs between

FIGURE 7.2
Creation of a composite between two varieties using controlled pollination and a combining ability trial



plants of the two entries. A full-sib test determines the specific combining ability of the pairs of plants that are crossed, whereas a topcross test determines the general combining ability of individual plants.

7.2.5 Recombination in selection cycles

Overview

Recombination in selection cycles differs from recombination to make the base population. The parents of the base population are diverse and unrelated, so crossing has to take this into account by using formal crossing designs. The parents for recombination during selection are all from the same population so all families are equivalent. Creating a base population requires a single random-mated population to be produced, whereas it is helpful during the recurrent selection cycles to also produce a family structure, such as full-sibs. Hence, in describing how recombination can be made during the selection phase, attention is paid to the resultant family structure.

Perfectly random recombination is where each individual plant produces half-sib seed by randomly crossing to the remainder of the population. However, in methods of recurrent selection where the test units are families (the only exception being mass selection), perfect randomness is undesirable as sibbing within families will cause inbreeding and create non-heritable between-plant variation. Natural outcrossing in pearl millet and outcrossing in maize, even when forced by detasselling, will produce half-sib families but cannot avoid within-family crossing as some of the pollen will unavoidably come from the same family as the female parent. Although certain planting designs will reduce this effect, it cannot be eliminated, particularly because within-family crossing inevitably occurs as a result of assortative mating caused by relatively higher intermating within early-flowering or later-flowering groups of families than among families with differing flowering times.

As a result of such limitations and the inefficiency of half-sib family selection, it is more cost effective to use forced crossing. The desirability of forced crossing increases the fewer the selected families and the more inbred they are. The forced intermating can produce topcrosses (individual plants crossed with bulk pollen) but sibbing among families is not completely avoided. Making full-sib families (individual plant × plant crossing) avoids sibbing entirely. Rope ladder crossing designs can be used to make this process more efficient (see below).

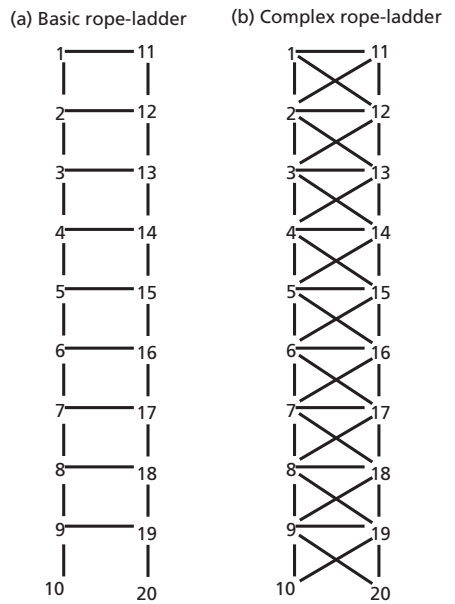
7.2.6 Production of full-sibs

The production of full-sibs provides the greatest control over pollination and avoids both within-family sibbing and selfing, but demands most labour. However, the increase in labour requirement to make full sibs is less than might be expected, because the work involved in making a pollen bulk by collecting and mixing aliquots of pollen is no longer required. A real disadvantage of full-sibs is that the effective population size is reduced over half-sibs because each entry in a full-sib nursery has only two parents, whilst a half-sib has many. The effective population size in the full-sib nursery can be maximized by using each plant in the full-sib mating only once as either a male or a female. Progress made by full-sib selection is expected to be much higher in the subsequent generation. Full-sib families have twice the additive genetic variance between them as half-sib families, making them more efficient as a selection unit, and the correlation between their performance *per se* (which is assessed in the trial) and their general combining ability (which determines their genetic value in the

next cycle of the population) is expected to be higher in full-sibs than in half-sibs unless very large dominance effects are present.

The production of full-sibs can be facilitated by using an appropriate planting plan (Figure 7.3) that avoids the complications of making the unnecessary full diallel between the selected families. 'Rope ladder designs' (Figure 7.3) can be used, and to increase the number of crosses that are possible using this design, the rope ladders are replicated. The number of replications required, r, is easily obtained from the specified number of crosses, n, and the number of selected families, s. After rounding up to the nearest whole integer r = n/s/1.5 (for the basic stepladder) and r = n/s/2.5 (for the complex stepladder). A random arrangement of the families within each replication is employed, and when the number of families is reasonably high there is little risk of repeating any particular cross to an excessive extent.

FIGURE 7.3
Mating designs for making full-sibs



Numbers indicate the entry number of a hill (or short row) planting, and lines indicate a cross that can be made in either or both directions. (a) gives 1.5 crosses per hill, and (b) gives 2.5 crosses per hill. (Modified from Geiger, 1985).

7.2.7 Improvement of base populations

Matching the selection environment to the target environment

In typical breeding programmes, selection in the segregating generations is conducted on-station in well-managed conditions. In many countries there are recommended packages of practices that require high standards of management and high levels of purchased inputs. They maximize yield, but farmers in marginal areas invariably apply lower inputs than are recommended for several good reasons: it matches better their limited capacity to procure resources, reduces their risks and maximizes their longer-term benefit:cost ratios by reducing or avoiding negative returns from purchased inputs in poor years. Hence, a common criticism of public sector-breeding programmes targeted at less favourable agricultural environments is that unrealistically favourable selection environments (SE) are employed (Almekinders and Elings, 2001; Ceccarelli, Grando and Booth, 1996; Virk *et al*., 2003; Witcombe *et al*., 1996). M. Bänziger (pers. comm.) has shown a typical mismatch between the SE and the target population of environments (TPE), where only the poorer research station environments match those of the farmers' fields (Figure 7.4). Hence, if the SE is to match the true TPE (Fischer *et al*., 2003) then the SE must not be optimal but encounter similar stresses to the TPE, such as low fertility and limited water.

Reducing the levels of on-station crop management can reduce the gap between the SE and the TPE. This approach has been used in client-oriented breeding programmes

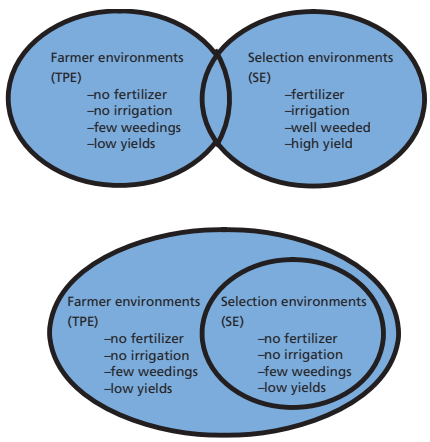
in maize in India, where the applied levels of fertilizer were significantly reduced from the recommended levels (e.g. Witcombe, Joshi and Goyal, 2003). A second strategy is to manage stress environments on-station to breed for tolerance to abiotic stresses commonly encountered in farmers' fields, such as drought and low nitrogen (Bänziger and Cooper, 2001). By testing progeny in both managed-stress environments and more favourable environments, families can be selected that not only tolerate these common stresses but also respond to more favourable environments. Since the stresses are managed, they can be carefully controlled so that the heritability of the trait under selection is increased and genetic gains are enhanced. A third strategy is to carry out selection in farmers' fields by breeders, or farmers, or both. If the selection programme is unreplicated (as is also typically the case on the research station), then a typical farmer needs to be chosen whose field is representative of the target area.

In more favourable agricultural environments the risk of a significant mismatch between the SE and the TPE is lower – both scientists and farmers manage the crop well, with applied, purchased inputs. Nonetheless, there are possible pitfalls. A mismatch still occurs when higher levels of purchased inputs are applied on the research station because, as in the case of marginal environments, the full economic cost and risk to farmers of applying them have not been considered. A mismatch can also result from disparities in the on-station cropping system used; researchers may employ more fallow or green manuring, because they are recommended rotations, even when farmers rarely adopt them (see also Chapter 6 in this volume).

Whether the selection is done by breeders, farmers, or breeders and farmers working together, will depend on circumstances (Witcombe *et al*., 2005). In many cases, it will be easier and more efficient for the breeders to do the selection, provided they have correctly identified the traits required by the target clientele. In some breeding methods that combine hybrid with OPV breeding, it makes no sense to involve farmers if most of the effort is on the development of inbred lines and on trials that assess their combining ability. In other circumstances, it may be easier for farmers to do the selection. In pearl millet, vom Brocke *et al*. (2002) argue that farmers' seed management practices can be incorporated into breeding programmes. Also in pearl millet, Monyo *et al*. (2000) described a breeding programme based on a farmers' deliberate selection within a cross the farmer had allowed to occur between the improved variety Okashana-1 and a local landrace.

If selection in the segregating generation is optional, it is essential to involve farm-

FIGURE 7.4
An idealized diagram of the gap between the selection environments and the farmers' environments (top) and the elimination of this gap (bottom)



Modified from M. Bänziger (pers. comm.).

ers in selection among the varieties that are produced by the programme. This process is commonly called participatory varietal selection (PVS) and involves farmers testing material in their own fields (Witcombe *et al*., 1996, 2005). The methods used involve some form of 'mother-and-baby' design (Snapp, 1999), where all of the entries are tested in relatively simple designs in the mother trials and subsets or individual entries are tested in baby trials. In this system, researchers and farmers evaluate the varieties. Particular attention is paid to the perceptions of the farmers for a range of traits and their overall preferences among the varieties.

7.3 POPULATION IMPROVEMENT METHODS REQUIRING ONE YEAR PER CYCLE

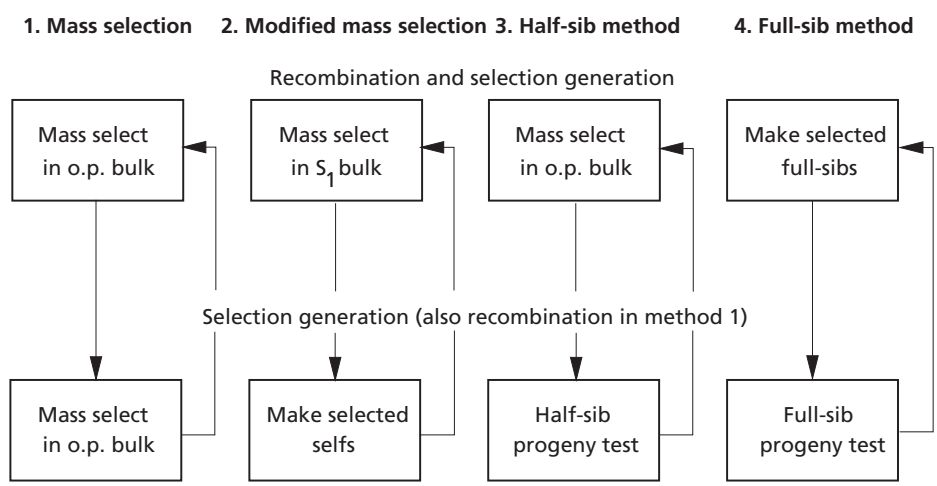
Mass selection, modified mass selection, half-sib family selection and full-sib family selection can be completed in a single year in locations where two generations can be grown in the field in a year (Figure 7.5).

7.3.1 Mass selection methods

General considerations

It seems to be an unavoidable fact that most plant breeders dislike simple methods. In self-pollinated crops most breeders employ elaborate forms of line selection whereas, when experimental comparisons are made, it is the simplest methods, such as bulk-population breeding or single-seed-descent methods, that show the highest efficiencies (e.g. Fahim *et al*., 1998, in rice). The same may apply to outbreeding crops where simple mass selection is rarely used, perhaps because it is considered to be an under-exploitation of the breeder's knowledge and skills. However, in outbreeding crops there is some justification for more elaborate methods because evidence shows that greater genetic gains per year can be made with progeny testing. Nonetheless, as can be seen below, significant genetic progress can be made from mass selection, and that progress requires the smallest investment in resources per unit of gain.

FIGURE 7.5
Four selection schemes that can be completed in a single year with two generations per year. (o.p. = open pollinated).



Because of this cost effectiveness and because of its simplicity, mass selection methods are attractive. They are particularly useful for decentralized breeding in difficult environments where complex breeding methods are beyond locally available capacity.

Mass selection is most effective when the initial base population yield is high and there is high genetic variance. Given these circumstances, high rates of genetic gain can be achieved in response to selection. As was discussed earlier, in the development of base populations, a combination of locally adapted material with high yielding exotic genotypes can produce this desirable combination.

Mass selection can also be made more attractive by simple modifications. The most commonly suggested modification is stratified mass selection, but personal experience has not shown this to be either simple to do or effective. Instead, alternative improvements to mass selection are considered after a brief consideration of the evidence of its effectiveness in pearl millet and maize.

7.3.2 Evidence of the effectiveness of mass selection

Pearl millet

Mass selection can be effective in improving pearl millet (Govil, Pokhriyal and Murty, 1986; Rattunde, Singh and Witcombe, 1989; Singh *et al.,* 1988). Rattunde, Singh and Witcombe (1989) showed in four composites that heritabilities from single plants were appreciable. Averaged across composites, the estimated heritabilities for 19 traits varied from 0.29 for yield, 0.45 for flowering time, to 0.64 for panicle length, indicating that mass selection will be effective for many important traits. Singh *et al.* (1994) demonstrated a significant gain from mass selection in the New Elite Composite (NELC) to produce a high-yielding open-pollinated variety, ICMV 155, that was released for cultivation in India.

Maize

Hallauer and Miranda (1988) report many studies on mass selection in maize in the 1960s and 1970s. The responses to mass selection were high given the simplicity of the method, but few of the experiments were conducted over more than three cycles. Examples of longer-term experiments are gains in grain yield of 19.1 percent per cycle over ten cycles (Genter, 1976) and gains of 2.1 percent per cycle over six cycles (Lonnquist, Cota and Gardner, 1966). More recent studies on mass selection are few. Weyhrich, Lamkey and Hallauer (1998) report on a comparison of selection methods in a population. Over ten cycles of mass selection an average gain of 0.6 percent per cycle was achieved, the lowest of the gains per cycle in the various selection methods (from mass selection to S_2). However, it was superior to half-sib family selection in gains per year. It was also the most superior of the methods in terms of cost-effectiveness; the costs per unit of gain were the lowest and the returns on investment the highest.

7.4 IMPROVEMENTS OVER SIMPLE MASS SELECTION

7.4.1 Gridded mass selection – do grids really help?

Various authors have suggested improvements over mass selection. Gardner (1961) developed an improved method of mass selection—gridded (or stratified) mass selection—and demonstrated its effectiveness in improving grain yield in maize. Burton (1974) reported on recurrent restricted phenotypic selection in Pensacola Bahiagrass (*Paspsalum notatum*) that differs from mass

selection by having five restrictions, the two most important being stratification (grids) as well as the control of pollination so that both male and female parents are selected (see below).

Rattunde, Singh and Witcombe (1989) examined heritability values in four pearl millet composites, using non-stratified and stratified data for many traits. Stratification was not worthwhile since the improvements in heritability with stratification were low and erratic. For stratification to be effective, uniform conditions are required within the strata, with a gradient in one or more environmental variables across them. In practice, such conditions seem to occur rarely in typical pearl millet experimental fields, which tend to be either uniform or have random variation. Experience of mass selection in maize has shown that this was a common problem in this crop as well.

7.4.2 Improving mass selection by simple modifications to selection procedure

Discarding poor areas of the plot

When the principles of client-oriented breeding are followed, only one base population is improved per target domain. Given the small number of base populations, this allows sufficient resources to grow them in large plots. Hence, it is possible to remove all of the plants before flowering from patches and field margins where the crop has grown poorly and still leave a large population.

Equal plant spacing

Equal spacing of plants eliminates an important source of environmental variability and improves the between-plant heritability. In maize, the population is hill planted (two plants per hill) and thinned to one plant per hill. In pearl millet, the crop can be sown at a more than adequate seed rate and then thinned to a uniform spacing.

Realistic selection differentials

In many mass selection schemes, very high selection pressures are applied by selecting, for example, the 100 best plants from 10 000 (e.g. Weyhrich, Lamkey and Hallauer, 1998). Instead, we apply strong roguing (removal of undesirable plants). Although the selection differential applied will be lower, it will be more reliable; when only a few phenotypically best plants are selected the risk increases that a high proportion of the selected plants are 'mistakes' because they happen to be in environmentally better situations.

Moving grids

The removal of the undesirable or poor plants is done by the breeder walking between alternate rows and removing plants in the rows on either side that are inferior to their neighbours. This can be likened to a form of 'moving grid' where selection is done on the relative performance of plants in a small area and better accounts for random (patchy) variation than grids.

Avoiding selfing

In mass selection in maize, the selected plants are allowed to random mate with the rest of the population. However, any maize breeder looking at the ear of a single white-endospermed maize plant grown amongst many yellow-endospermed plants (or vice versa) cannot fail to be surprised by how numerous are the grains with the maternal grain colour that result from selfing. Even though reports in the literature report selfing rates of about 5 percent, this is not based on any extensive experimental data. The proportion of selfing may be considerably higher, particularly when wind speeds

are low during anthesis. Because selfing may be high it makes good sense to see that the population to be mass selected is entirely outcrossed. The avoiding of selfing means that the mass selection is more efficient. Even with only 5 percent outcrossing, 5 percent of the plants have to be removed for inferior phenotypic performance because of inbreeding, without any genetic gain being made, and with higher selfing rates the problem increases.

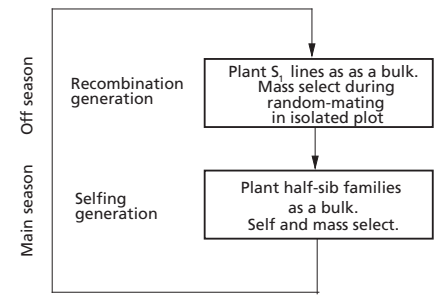
Selfing is avoided by detasselling 50 percent of the population and after mass selection (which is done on all of the population of plants whether detasselled or not) only the ears from the detasselled plant contribute to the next generation. The detasselling does not add greatly to the labour involved as selection, which has to be done frequently to eliminate poor pollinator plants as early as possible, can be combined with detasselling. Alternatively, unskilled workers can be employed to do the detasselling.

Selection before pollen shed

Frequent, early selection, rather than selection just at maturity, means that many plants can be eliminated before pollen shed. Hence, selection is exerted not only on the female parent but to a lesser extent on the male parents as well (so $c > 0.5$ but < 1 in the equation below).

One form of modified mass selection is to employ alternate selfing and recombination generations (Figure 7.6). This is feasible in pearl millet as selfing only involves bagging panicles, whereas controlled crossing between the tassel and silk is required in maize. The major advantages of employing alternate selfing generations is control of pollination in the selfing generation, which doubles the efficiency of mass selection as it is applied to both the male and the female parents. In the subsequent recombination generation, higher selection efficiency is achieved because the between-plant heritability between S_1 plants is higher than between S_0 plants.

FIGURE 7.6
A scheme for modified mass selection



The scheme takes one year per cycle with two cropping seasons per year

Of practical interest is the question of in which generation is selection the most efficient? Predicted gain with varying degrees of pollination control can be estimated from the following equation (Hallauer and Miranda, 1988):

$$\Delta G = ic\sigma_A^2 // \sigma_A^2 + \sigma_D^2 + \sigma_E^2$$

where: i is the selection intensity or standardized selection differential; and c is a coefficient where $c = 0.5$ (no control over pollination and selection after pollination) or $c = 1$ (control over both male and female gametes as in the case of selfing). The remaining terms refer to the square roots of additive (σ^2A), dominance (σ^2D) and environmental (σ^2E) variances. Selfing controls the source of both male and female gametes ($c = 1$). In simple mass selection, the selection takes place after pollination, so selection is only on the female parent ($c = 0.5$). Hence, controlling pollination by selfing always doubles the selection efficiency over simple mass selection.

The efficiency of selection in the S_1 bulk is increased over simple mass selection by

1.2 to 2.4 (Dhillon, 1991). This is because the inbreeding caused by selfing increases the additive genetic variance between plants and recessive alleles are more frequently expressed phenotypically. However, values that are at least 2.0 (note 2.0 is always achieved in the selfing generation) are rare and occur only when dominance is high (the additive genetic variance increases more) and when gene frequency is high (for the same reason). Hence, under most circumstances the selection efficiency will be highest in the selfing generation and it makes sense to grow this generation, rather than the S_1 bulk, in the season where the crop is most frequently grown. Further justification comes from studies in maize that showed that the correlation between performance *per se* of the S_1 plants (on which selection is based), and their general combining ability (that determines their contribution to the performance of the population), is not as high as theoretically expected (reviewed by Seitz, 1989). This reduces the expected efficiency of selection amongst S_1 plants.

7.5 HALF-SIB METHODS

7.5.1 Overview

We strongly recommend to any maize or pearl millet breeder not to use half-sib family methods of selection. The theoretical advantages of half-sib family selection are limited and experimental evidence supports the theory. The possibilities are too great of being misled by the effects of non-random mating in the production of half-sibs to make the method attractive. If the degree of selfing was higher in the crossing block in some half-sib families than in others (a likely occurrence because selfing will be higher in early and late flowering plants) then the non-heritable variation in between family means will be high.

7.5.2 The method

The genetic variance among-sib families accounts for only 1/4 of the additive genetic variance, whilst the remainder is within them. Hence, most of the selection has to be exerted within families where it is equal to the efficiency of simple mass selection. Unfortunately, many of the modifications to simple mass selection suggested above will not be practical in the context of a half-sib family trial.

The selection efficiency, however, is not entirely related to the proportions of additive variance among and within families. Entries can be replicated in the progeny trial to increase the heritability of the half-sib family means, whereas no replication is possible for the selection of individual plants within the families. However, since within-family selection is essential, no matter how many replications are used, it is desirable to have spaced plants that demand additional land. Once spaced plants are combined with replication of families the method becomes resource demanding.

7.6 FULL-SIB METHOD

Schipprack (1992) has discussed the high efficiency of full-sib selection compared with other methods. One cycle can be completed in two generations in a single year (Figure 7.4). This progeny testing method allows one cycle per year where the families are evaluated in the field and, unlike the case of half-sib families, the additive genetic variance between families is at least as high as the additive genetic variance within them. The full-sib method does not require an isolated plot for recombination. However, hand control of pollination is required to make crosses and, in pearl millet, this needs more labour than making selfs, whereas in maize the labour requirements are almost the same. There has to

be a sufficient number of these crosses to avoid inbreeding in later cycles.

7.7 METHODS REQUIRING INBREEDING

In general, if the breeding of open-pollinated varieties is not combined with the breeding of hybrids, then any method that goes beyond the S_1 stage will not be efficient. The correlation between performance *per se* and general combining ability worsens with the degree of inbreeding. In methods that use inbred lines to make OPVs (Bänziger and Cooper, 2001) then combining ability trials of the inbred lines are required. This is efficient if hybrids are being bred, but much less so if the purpose is only to breed OPVs.

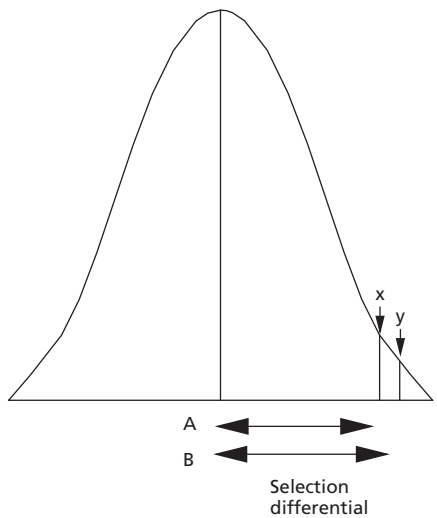
However, in all selection methods where there is no inbreeding (mass, half-sib and full-sib methods), selection for dominant alleles is less efficient compared with when some degree of inbreeding is used (e.g. S_1 or S_2). In pearl millet, when selecting for resistance to downy mildew, which is a dominant trait and determined by loci of large effect (Jones *et al.*, 1995), the efficiency of full-sibs as a test unit is lower than that of S_1 lines. With loci of large effect and dominant gene action, full-sib and S_1 testing can be directly compared from the predicted gains from these two methods (Hallauer and Miranda, 1988) without the need to determine the expected correlation between the performance of S_1 lines *per se* and their general combining abilities. The smallest disadvantage for full-sib selection will be when the recessive susceptible allele is at intermediate frequencies, but its greatest disadvantage will be when the allele is at a low frequency, since inbreeding will be required to uncover it. A good strategy, therefore, in breeding for disease resistance controlled by major resistance genes would be full-sib selection in the early stages when the frequency of recessive alleles is high, followed by S_1 selection when some progress has been made in reducing the frequency of the susceptible alleles.

7.8 PRODUCING VARIETIES

The underlying theory behind the production of varieties involves two main parameters, the selection differential that can be applied to the entries in the progeny test, and the resultant inbreeding depression of the variety. A higher selection differential is applied to make a variety by selecting fewer lines than when the next cycle of the composite is recombined. The higher yield and reduced genetic variance expected from using fewer lines is desirable in a variety. However, the difference between the selection differential applied to produce a variety and to produce the next cycle bulk is inevitably small (Figure 7.7). Moreover, if a high selection differential is to be applied to making varieties, then even different subsets of entries selected according to varying criteria, such as location-specific adaptation, will inevitably have in common entries that are high-yielding in all locations. Greater genetic gains can be made by making and improving new composites than by increasing the number of varieties made from the same cycle of a composite. Consequently, only one or very few varieties should be made from every cycle of the composite.

As a small number of varieties (1 to 4) are made each cycle, larger numbers of selected families (10 to 25) are used to make varieties. Larger numbers give a more predictable selection response since sampling error is reduced; the smaller the number of lines the greater the proportional contribution of any line misidentified as high-yielding because of experimental error. The reduced selection differential that is applied

FIGURE 7.7
An illustration of the small differences in selection differential that can be applied when selecting families to produce the next cycle bulk or variety



Means of the selected lines (x) and the selection differential (A) for the next cycle bulk, mean of the selected lines (Y), and selection differential (B) for a variety.

when a larger number of lines are selected is compensated, at least in part, by the reduced level of inbreeding expected.

Inbreeding increases when fewer lines are used for making a variety. It also increases with the degree of inbreeding of the selected lines, since the more inbred they are, the greater the expected inbreeding of a variety made from them.

For S_1 lines, about 15 is optimal (Busbice, 1969), and in practice this gives good results. Since most composites are created and selected to have distinct morphological and phenological characteristics, the larger numbers of lines do not cause undesirable increases in variability.

Mass selection avoids any inbreeding caused by selecting a subset of lines, and avoids the error involved in selecting only a few of them. In pearl millet, several high-yielding varieties have been bred from ICRISAT composites by mass selection within a population, of which the released variety ICMV 155 is one example (Singh *et al.,* 1994).

7.9 SUMMARY AND CONCLUSIONS

In the formation of composites, choice of initial parental material is dependent on the intended use of the population. Parents can be carefully chosen by understanding the needs of the clients. These can be determined by using farmer-participatory approaches. Information on farmers' selective adoption and the results of PVS trials can be used to identify useful parents. A compromise has to be achieved between high initial yield and genetic variance, and the number of parents used will vary greatly according to this compromise

Methods of making the initial cycle bulk vary from the simple to the elaborate. Ideally, the method should allow a visual assessment of the degree of recombination. When there are not too many parents, it is most efficient to use some form of diallel crossing in the first generation of random mating. Combining ability can be tested when forming a new base population by combining two varieties or populations.

Recombination of selected entries from the progeny trials during recurrent selection to produce half-sib families is fraught with difficulties, and makes the production of full-sibs an attractive alternative. This has the added advantage of providing a more efficient test unit in the subsequent generation.

Particular attention is paid to population improvement methods that take one year per cycle. Selection is carried out in an appropriate environment for traits that are important to the target group of farmers. Appropriate environments can be achieved

by making the research station environment match those of farmers' fields. Alternatively, selection can be decentralized to the farmers' fields. Managed stress nurseries can be used to select for abiotic stresses commonly encountered in the farming systems of the client farmers.

Plant breeders often disregard simple methods in favour of the more elaborate, even when simpler ones are more cost effective. Mass selection is very cost effective and there are many simple ways of increasing its effectiveness. In maize, these include the complete avoidance of selfing by detasselling to eliminate the errors involved in selecting among plants with differing degrees of inbreeding. In pearl millet, they include alternate selfing and recombination generations to allow control of pollination and to increase between-plant heritability.

Of the methods that use family testing, half-sib methods are theoretically the least efficient and experimental evidence supports this. The full-sib method is the only one that permits a field test with families that have a high between-family additive genetic variance, and completes a cycle of selection in only one year. Methods that involve a degree of inbreeding, such as S_1 and S_2, are best combined with hybrid breeding. However, even in the breeding of OPVs, S_1 testing can assist in the fixation of dominant alleles that determine disease resistance.

The theory relevant to the production of varieties from composites indicates that differences in yield between varieties will not be high, since all of them will be created employing similar selection differentials. A minimum number of entries are required to make a variety to avoid inbreeding depression and reduce the sampling error involved in the selection. Mass selection from the source population is a simple and effective way of making varieties.

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CHAPTER 8

Methodologies for generating variability Part 4: Mutation techniques

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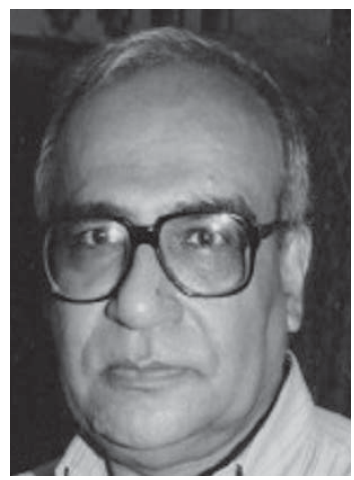


8.1 INTRODUCTION – ECONOMIC IMPACT OF INDUCED MUTATIONS

The use of various mutagens to generate genetic variation in crop plants has a history almost as long as that of conventional breeding. Induction of variability by irradiation of barley seeds with X-rays was already demonstrated in 1928 by Stadler. The application of this phenomenon has come a long way to become a real tool, not only in crop breeding but also in basic research on the plant genome, its structure and function. Breeders were the first to recognize the potential of induced mutations through analogy with spontaneous mutants, often selected as new plant types in many crops, from cereals to apples, not to mention ornamental and decorative plants. Many mutants with desired traits were selected in the second or third generation after mutagenic treatment and subsequently released as new cultivars after agronomic evaluation in regional and national trials. These or other mutants developed with mutations in desired traits, even though not released as new cultivars, have been used in cross-breeding programmes as a source of particular alleles, often allelic to the spontaneous ones, but in a desired genotype. Among them were sources for characters such as short stature and lodging resistance; disease resistance; oil quality; and increased nitrogen fixation. These mutated genes are especially valuable as the best currently grown cultivar was usually selected for mutagenic treatment. A desired mutation in a good genetic background is a very attractive component in breeding programmes. This approach is much simpler and faster than crossing with an exotic source, and it is one of the main reasons for the wide use of mutated alleles in the breeding of numerous species.

8.2 CULTIVARS OBTAINED BY MUTATION BREEDING

By 2000, the FAO/IAEA Mutant Varieties Database (MVD) had collected information on 2 252 cultivars obtained by mutation and officially released in 59 countries worldwide, mainly in Asia (1 142), Europe (847) and North America (160). Almost half of these cultivars (1 019) were released after 1985. The list of crop species with induced mutant cultivars reached 175 in 2000, compared with 154 crop species in 1995, which indicates increased dynamics in the contemporary application of this technique in plant breeding. This list included many important crops, including rice, wheat, cotton, oilseed rape, sunflower, sesame and grapefruit. Of the 2 252 cultivars, 75 percent (1 700) are in crops and the rest (552) in ornamental and decorative plants. In sexually propagated crops, with 1 603 mutant cultivars released, cereals (1 072) dominate, followed by legumes (311), industrial crops (81), vegetables (66), oil crops (59) and others (111). About 70 percent of cultivars from this group were released as direct mutants; the remaining 30 percent were developed as recombinants from crosses with mutants. Of 1 585 directly developed mutant varieties, the great majority (1 411) were selected from mutated generations following the use of radiation, mainly gamma rays, as the mutagen (Maluszynski *et al*., 2000).

Most of the desired genetic variation explored in breeding programmes has occurred naturally and is preserved in germplasm collections. However, when these collections fail to provide a source for a particular trait, it is necessary to resort to other sources of variation. In such cases, mutation techniques provide tools for the rapid creation of desired traits. Even though the great majority of induced mutations are recessive and deleterious from a breeder's

point of view, it is possible, with proper selection tools, to find desired genotypes from adequately large mutated populations. As a result of these unique possibilities, mutation techniques have significantly contributed to plant improvement worldwide, and have had an outstanding impact on the productivity of some crops.

Rice

Among 434 mutant cultivars of rice, cvs. RD6 and RD15, developed after gamma ray treatment of an old local variety, KDML105, have had enormous economic impact. They were released in Thailand in the late 1970s. According to the Bureau of Economic and Agricultural Statistics in Bangkok, during 1989–1998 these two varieties were planted on a total of nearly 24 million hectares and yielded 42 million tonne of paddy, or 26.9 million tonne of milled rice, worth US$ 16.9 billion. More than 20 years after their release, both varieties are still grown extensively in Thailand. The gamma ray-induced sd_1 mutation, allelic to the spontaneous 'Green Revolution gene' sd_1 in rice, led to the release of the mutant cultivar Calrose 76 in California in 1976 (Rutger, 1992). This mutated gene has been extensively transferred by crosses into other genetic backgrounds, which resulted in the release of 20 new cultivars in countries on three continents. This includes the leading Australian mutant cv. Amaroo, released in 1987. This semi-dwarf cultivar covered 60-70 percent of the rice cultivation area, and on average yielded 8.9 t/ha. Gamma ray-induced cv. Zhefu 802 was the most extensively planted conventional rice cultivar in China between 1986 and 1994. This variety has a short growing period and high yield potential even under infertile conditions and poor management, which contributes to its wide adaptability.

Barley

In barley, two short-stature mutant cultivars have made a major impact on the brewing industry in Europe: cv. Diamant, released in 1965 in the former Czechoslovakia, and cv. Golden Promise, developed in Scotland in 1966 following gamma ray treatment. Both have added billions of dollars to the value of the brewing and malting industry. The X-ray-induced gene *denso* from Diamant, allelic to some spontaneous sources for semi-dwarfness, has been transferred to about 180 cultivars in Europe and other continents. Cv. Golden Promise has stiff straw, high yield and improved malting quality. It was widely used in the UK and Ireland for the production of whisky and beer. This variety contributed US$ 417 million to grain production in Scotland between 1977 and 2001. It is still popular for its high quality. It was recently discovered that Golden Promise is also salt tolerant. The FAO/IAEA MVD listed 269 barley mutant cultivars officially released in 28 countries (MVD, no date).

Wheat

In wheat, there are 197 officially released mutant cultivars. The most impressive is the mutant cv. Creso, released in 1975 in Italy. This durum wheat variety was obtained by crosses with a semi-dwarf mutant from variety Capelli, following thermal neutron treatment. Creso was grown on 400 000 ha and shared 53.3 percent of the market of certified durum wheat seeds in Italy as early as 1984. The estimated additional grain yield over the decade 1983–1993 of its cultivation was valued at US$ 1 800 million. The gene for semi-dwarfness from Creso is still used in breeding programmes in Italy, Austria, Bulgaria and other European countries where durum wheat is cultivated. More recently, due to some problems

with fertility of bread wheat cultivars with semi-dwarfness genes *Rht-B1* and *Rht-D1* in temperate climates, the mutant line 'Krasnodarskii karlik' with gene *Rht11* has become more widely used in cross breeding programmes in Europe and Australia.

Cotton

In cotton, mutagenic treatment with gamma rays led to the development of two very important cultivars – NIAB 78 in Pakistan and Lumian No. 1 in China. NIAB 78 was released in 1983 in Pakistan and during the following ten years it doubled cotton production - contributing more than US$ 3.0 billion. The added income to cotton growers due to the cultivation of this cultivar from the year of its release onwards has been estimated at US$ 486 million. NIAB 78, due to its wide adaptability, tolerance to heat and escape from bollworm attack because of early maturity, saved the textile industry of Pakistan, which was threatened by reduced cotton production. Annual cultivation of the high yielding mutant cultivar Lumian No. 1 exceeded one million hectares by the late 1980s. It was the most widely grown cotton cultivar in China.

Vegetatively propagated crops

Important results have also been obtained in breeding vegetatively propagated crops. Two outstanding examples are mutant cultivars of grapefruit and of Japanese pear. Budwood of grapefruit mutant cv. Star Ruby irradiated with thermal neutrons led to the release of the 'Rio Red' cultivar. This mutant cultivar was released in 1984 in Texas. It is seedless and has red flesh, red and stable juice colour, and good yield. The fruits of these cultivars are known under the trademark 'Rio Star'. They are grown on 75 percent of the grapefruit-producing area in Texas. The mutant cultivar 'Gold Nijisseiki' of Japanese pear was developed with chronic irradiation in the gamma field in Japan. The cultivar is more resistant to Black spot disease and needs only one or two applications of fungicides per season. The additional annual income for growers is almost US$ 30 million (Ahloowalia, Maluszynski and Nichterlein, 2004).

8.3 MUTAGENIC TREATMENT

8.3.1 Radiation

The FAO/IAEA Database on Officially Released Mutant Varieties (MVD, no date) indicates that radiation, especially gamma rays, has been the most often used treatment for inducing mutations of crop plants. The reason for this is the simplicity of the treatment rather than to any higher efficiency of mutation induction. Seeds or other organs of the plant have to be delivered for irradiation to a nuclear centre. Such centres have been established in most countries. Nuclear centres are usually supervised by the Ministry of Energy or directly by the Prime Minister's Office. They will inform the plant breeder which centre is providing a 'seed irradiation service'. Mutagenic treatment is free of charge in most developing countries. Dry seeds (M_0 generation), disease free, with good germination ability and about 12–13 percent moisture content, can be sent for irradiation by regular mail. As the process of acute irradiation is very short, they should be returned to the breeder in the same way – by mail, within one or two weeks. This is possible because irradiated seeds (M_1 generation) or other plant organs are not radioactive and can be used directly for sowing or kept refrigerated awaiting the proper sowing period, even for a few months, depending on the crop species. A free-of-charge seed irradiation service is also provided by the FAO/IAEA Agriculture

Laboratory, Plant Breeding Unit, A-2444 Seibersdorf, Austria (<*Official.Mail@iaea.org*>). Any plant breeder can send seeds for gamma ray irradiation to this address. The scientists working there can advise on the dose of gamma rays or apply the dose requested by the breeder. They will also adjust seed moisture content if necessary.

Physical mutagens are also very useful for inducing mutations in vegetatively propagated crops and in *in vitro* cultures. Cuttings, immature spikes or Petri dishes with explants, calli, somatic embryos or microspores are often subjects of irradiation by ultraviolet (UV), gamma or X-rays.

8.3.2 Chemical mutagenesis

The use of chemical mutagens is also very simple and can be done in any biological laboratory with basic equipment. However, it should be kept in mind that most chemical mutagens are also strong carcinogens. For this reason, all steps of mutagenic treatment should be carried out wearing gloves and under a Biohazard flow-hood. These safety conditions are not necessary for treatment with sodium azide, which is a very powerful mutagen, but only for a limited number of species, including barley, rice, maize, oat, sorghum, sesame, jute and soybean. Numerous chemical mutagens have been successfully used for crop improvement (Table 8.1).

The mutagenic action of a chemical mutagen induces somatic and genetic effects in a treated cell, tissue or organ. After treatment of seeds, only unrepaired damage to the DNA in initial cells of the sporogenic layer (germline cells) are transferred as mutations to the next generation. Other mutations in somatic cells of the embryo, including mitotic chromosomal aberrations, together with toxic action of a mutagen on all components of cytosol, affect plant growth and development, and are called the 'somatic effect' of the mutagen.

The steps generally followed in mutagenic treatment of seeds with chemical mutagens are:

- pre-soaking in distilled water;
- pre-treatment rinsing in tap water;
- treatment with the mutagen;
- post-treatment rinsing in tap water; and
- drying (if necessary) on a filter paper.

All steps of mutagenic treatment should be done using glass beakers to avoid any interaction of chemical mutagens with even trace quantities of metallic cations or other active reagents. Seeds for each dose of mutagenic treatment (M_0 generation) and for the untreated control—usually the parent variety—are put into beakers that are visibly labelled with the applied concentration of mutagen.

As dry seeds are usually used for treatment, pre-soaking in distilled water should

TABLE 8.1
Chemical mutagens used most commonly in plant mutagenesis

Name	Abbreviation	Molecular weight[1]
Ethyleneimine	EI	43.07
Dimethyl sulfate	DMS	126.13
Diethyl sulfate	dES (DES)	154.19
Ethyl methanesulphonate	EMS	124.20
N-ethyl-N-nitrosourea	ENU (ENH)	117.11
N-methyl-N-nitrosourea	MNU (MNH)	103.08
N-methyl-N[1]-nitro-N-nitrosoguanidine	MNNG	147.09
Sodium azide	NaN3	65.01

Notes: (1) data from SIGMA, 2005

be applied to activate seeds physiologically before treatment with mutagen. The amount of water used in pre-soaking should be at least two to three times the volume of dry seeds. The beakers with pre-soaked seeds should be gently shaken a few times to remove air bubbles, which can block access of mutagen to embryos. Duration of pre-soaking depends on the biology of germination of a particular crop species. For example, in barley and other major cereals, 8–10 hours of pre-soaking in room temperature (20–24°C) is usually applied. Pre-soaking significantly reduces the somatic effect of chemical mutagen. Short washing, 2–3 times in room-temperature tap water should be applied after soaking to remove water-soluble substances leaching from the seed.

Such prepared seeds are ready for mutagenic treatment. It is advisable to use three doses of mutagen for a large-scale field experiment. This is especially desired for regions with very variable and unpredictable weather conditions during the growing period of mutagenetically treated material. Drought, cold and heat can significantly modify the somatic effect of a mutagen and influence the final effect of treatment.

The concentration of mutagen, its duration and temperature of treatment are understood under the term 'dose' in chemical mutagenesis. A temperature of mutagenic solution of 22–24°C is most often applied for the seed treatment of various crop species. The use of other temperatures is also possible. However, it should be noted that the increased temperature will significantly shorten the half-life of chemical mutagen and generate products of hydrolysis that can increase undesired somatic effect of a mutagen. This is especially relevant to treatment with mutagens such as dES or EMS. To obtain equal penetration of a mutagen through the cells of a seed embryo, it is necessary to treat seeds in a water solution of the mutagen for 3 to 5 hours. Similar to the pre-soaking, the treatment should be done with a significant surplus of mutagenic solution, some 2 to 3 times the volume of the dry seeds. In cereals, about 1–1.5 ml of mutagenic solution is applied per seed. The concentration of the mutagen should be considered, together with duration of the treatment. A shorter treatment time with higher concentration of mutagen can increase somatic effects and could be insufficient to penetrate equally all cells in the plant material. A gentler treatment requires a lower concentration but longer period of application.

Extensive post-treatment rinsing several times in room-temperature tap water is necessary to stop action of the mutagen and to remove its residues from the surface of the seeds. To facilitate sowing, the treated seeds can be dried on filter paper under a fume hood. However, too intensive drying, especially with increased air temperature, can enhance somatic effects of the mutagen. Surface-dry seeds are ready for sowing and are termed the M_1 generation. In a well organized laboratory, pre-soaking is done overnight and mutagenic treatment in the early morning. This allows the M_1 seeds to be sown the same day. Should this be impossible, due to prolonged pre-soaking or mutagenic treatment, the mutagen-treated seeds, after brief drying, can be kept in a refrigerator at a temperature of around 6 to 8°C.

Some mutagens are active in a particular acidity of a treatment solution. This is the case for sodium azide, which is a very efficient mutagen in several species if applied at low pH. For this reason, sodium azide is dissolved in a phosphate buffer at pH 3 and this solution is used for treatment.

8.4 INDUCED MUTATIONS IN CROP PLANTS

8.4.1 Determination of treatment dose

Choosing the treatment dose is probably the most important decision in mutation breeding or genomic research using induced mutation. As the selection of mutants is done in large mutated populations, any mistake in choosing the right dose of mutagen will determine the success or failure of the entire breeding programme. The description of generations after mutagenic treatment is given in Figure 8.1. Before deciding on the use of mutation techniques, a number of parameters need to be carefully considered, and they are discussed below.

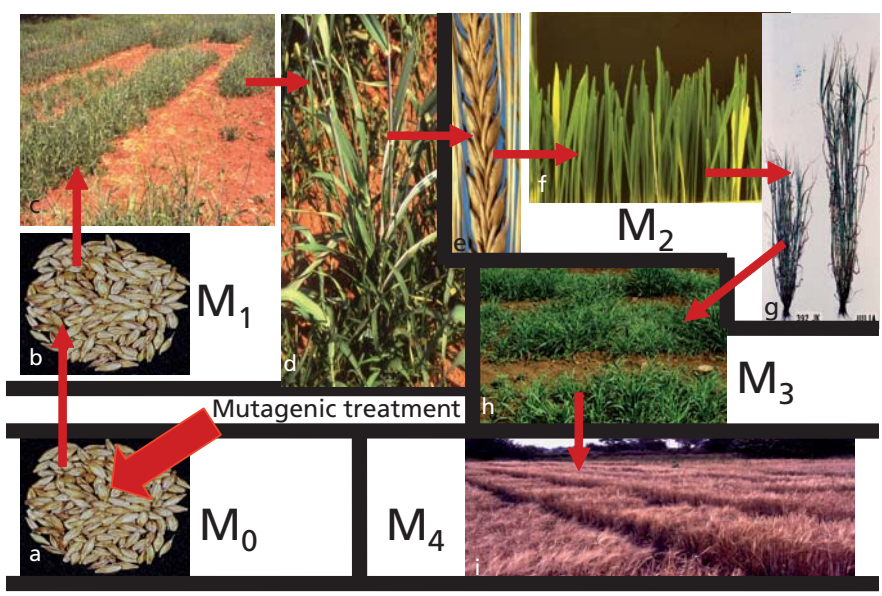
The objectives

These can be divided into two groups. The first is the programme of improvement of one particular character in a promising cultivar or breeding line, and is most often chosen for the direct release of desired mutant line as a new cultivar, without the use of a cross-breeding approach. The second programme deals with basic research or with the development of new gene sources, not present in available germplasm, which have to be transferred by crosses into other breeding lines.

The plant material

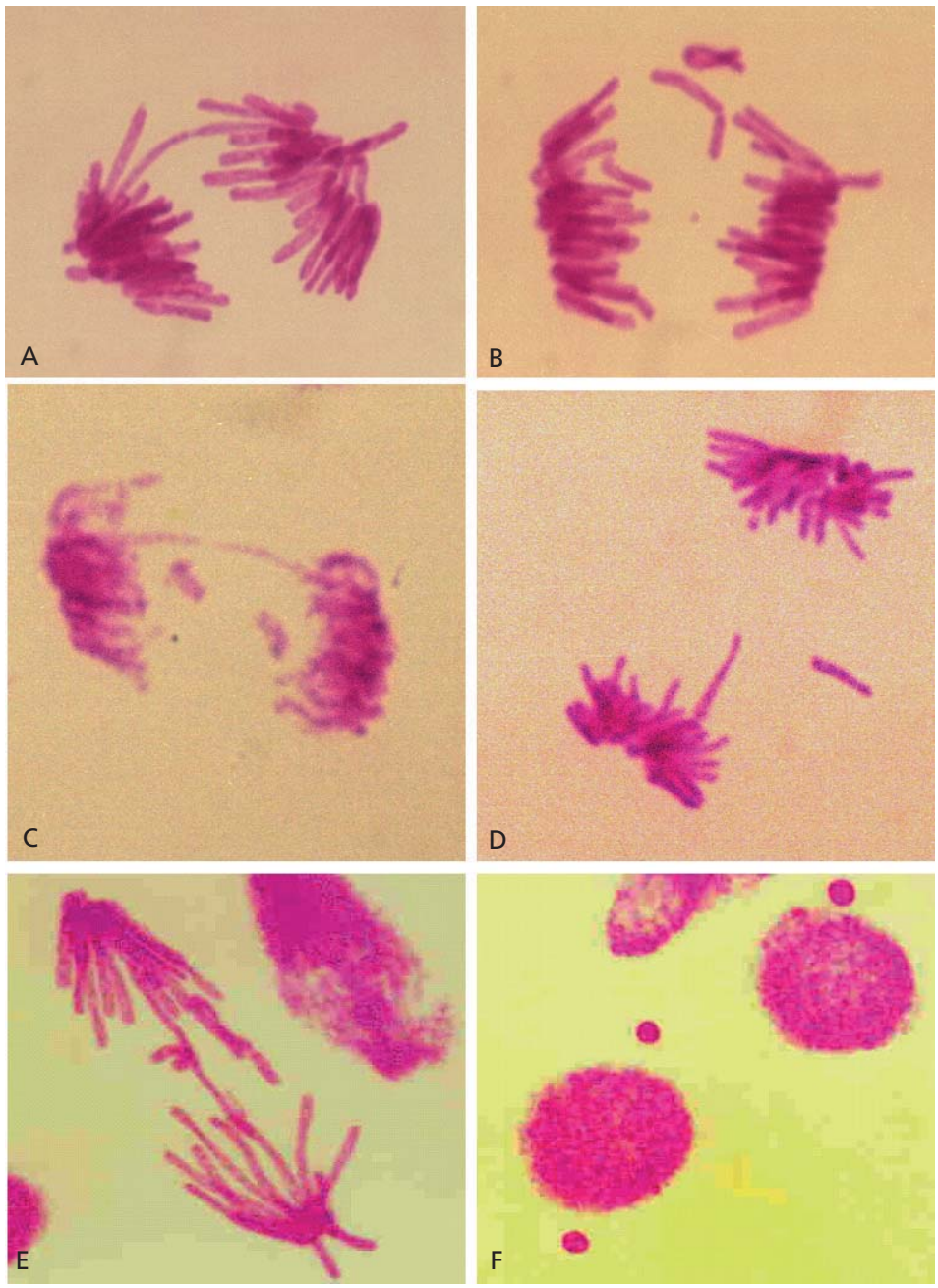
The objective of induced mutations determines the plant material. Contrary to basic research on plant mutagenesis, where homogenous and homozygous lines are preferable, various breeding materials have been successfully used for mutagenic treatment to obtain new, improved cultivars. Most often, already released cultivars are

FIGURE 8.1
Schema of induced mutations project with description of mutated generations



Notes: a) seed before mutagenic treatment – M_0 generation; b) the same seed after mutagenic treatment – M_1 generation; c) M_1 generation in the field; d) individual M_1 plant with visible chimeric chlorophyll sectors; e) spike of M_1 plant with M_2 seeds; f) M_2 chlorophyll mutants at seedling stage; g) mutant selected in M_2 generation and its parent variety; h) M_3 generation; i) small plots of selected mutants in M_4 generation.

FIGURE 8.2
Structural chromosomal aberrations in anaphase of root meristems



A–B *Vicia faba* and C–D *Hordeum vulgare* after maleic hydrazide treatment; E–F *Vicia faba* after MNU treatment; a) bridge; b) two fragments; c) bridge and two fragments; d) fragment and delayed chromosome; e) bridge and numerous fragments: f) interphase with micronuclei *(courtesy of Dr. J. Maluszynska).*

mutagen-treated, to improve one or two characters that can significantly increase their agronomic value. Very often, promising breeding lines, F_1, F_2 and later generations of various crosses, doubled-haploid (DH) lines or even natural populations, have been used as material for treatment.

The mutation frequency of the gene responsible for desired character

Knowledge of the natural mutation frequency of the gene responsible for a desired character will help very much in choosing the mutagen and its dose. In the case of inducing short stature in rice and other cereals, a population of 10 000 to 30 000 M_2 plants is usually sufficient to find a desired phenotype. Unfortunately, in most cases, the frequency of mutation at the locus of interest in particular species is unknown, or a previously used mutagen unavailable. However, it should be clearly remembered that the frequency of mutations observed in one species or even in another cultivar of the same species differs markedly from another cultivar or species. The induction of mutation has two major steps: damage of DNA; and its subsequent repair. Both these processes, and especially DNA repair, depend on many cellular factors involving numerous enzymes. Diversity of the DNA repair machinery, among other factors, generates the variation in somatic and genetic effects of mutagenic treatment.

Somatic effects of mutagenic treatment in M_1

As a large mutated population at the M_2 or M_3 stage is necessary to select the phenotype desired, the level of somatic effects in the M_1 generation determines the amount of mutated seeds which can be used in the next generations. The level of somatic effects after mutagenic treatment can be evaluated on the basis of various parameters, including delay in seed germination; level of disturbances in the cell cycle; frequency of chromosomal aberrations in meristematic tissues (Figure 8.2); reduced seedling emergence; reduced seedling and plant growth; appearance of chlorophyll defects; and reduced fertility and plant survival. The term 'reduced' indicates change in expression of a particular character in relation to the control, usually the parent cultivar or the breeding line whose seeds were treated with a mutagen. The reduction is expressed as a percentage. In other words, the control has 100 percent of the value of any parameter and 0 percent of its reduction. It should be noted that all steps of mutagenic treatment should be the same for treated material and for parent variety, except for the use of mutagenic solution, which should be replaced by distilled water or pH buffer, if used.

Desired and possible size of the M_1, M_2 and M_3 populations

The size of the M_1 population is rather small in comparison with the following generations. In cereals, a few thousand seeds per treatment dose should be enough to obtain 10 000 to 30 000 seeds for the M_2 generation, if the applied mutagen dose was not too high. For other crops, the knowledge of seed production by an individual plant of the parent variety in the particular experimental field is very helpful for this calculation. The field size of M_2 and M_3 generations, together with the degree of difficulties in selection techniques, in great part determines the cost of the programme.

Selection technique to be applied

The change of a morphological character is most often the subject of selection. Easily visible characters such as plant height, til-

lering, flower and fruit shape and colour, but also resistance to herbicides, allows the screening of very large populations under field conditions. What is usually not possible or too costly is selection using any laboratory technique.

In summary, the chosen doses for mutagenic treatment should be relatively low for the improvement of a parent material with favoured genetic background. It should be noted that mutagenic treatment generates mutations in many other genes in the genome of each cell, not only at the desired locus. One result of using too high a dose is a high frequency of desired mutations, unfortunately also accompanied by a high frequency of deleterious mutations in other important loci. The deleterious mutations negatively influence the agronomic value of selected mutants. Such mutants selected in M_2 or M_3 generations usually have problems of low fertility, late maturity or susceptibility to stresses, i.e. the characters that directly influence yielding capacity. The use of high doses of mutagens, in the era of application of induced mutations termed as 'mutation breeding', was the reason that many programmes in the 1960s and 1970s did not yield the expected results. A dose causing 50 percent lethality (LD_{50}) was often suggested as the optimal for breeding programmes, resulting in too high a frequency of deleterious mutations. Nevertheless, it should be noted that low doses of mutagen decrease the frequency of mutations and in consequence a larger population of M_2 or M_3 is necessary to find the most desirable mutants in a promising genetic background. It is a good breeding practice to cross a selected mutant with its parent variety and select desired recombinant from the segregating F_2 generation. This approach, known as the 'cleaning method', helps in elimination of undefined deleterious mutations from a mutated genotype. Significant yield improvement of the selected recombinant with the mutated phenotype in relation to the original mutant is the best illustration that the elimination of deleterious mutations has been achieved, at least partly.

For the breeder without experience in the use of mutation techniques, the most difficult problem is to identify, in practice, a proper dose for mutagenic treatment, keeping in mind the considerations discussed above. There are several laboratory tests to help define a critical dose for both physical and chemical mutagens. The term 'critical dose' implies the dose of a mutagen beyond which the somatic effects in the M_1 generation are too high. In sexually propagated crops, doses of LD_{50} and above are definitely considered to be critical doses in current approaches to induced mutations for breeding purposes.

The simplest and cheapest laboratory test, suitable for the evaluation of somatic effects of mutagenic treatment in mono- and dicotyledonous crop species with any size of seeds, is a pot test for the measurement of emergence and seedling growth reduction. To perform this test, ceramic or plastic pots, 18–22 cm diameter, are all that are needed. The pots can be replaced by any other plastic container of a similar volume and size. Metal and wood containers should be avoided as they can release ions or active reagents influencing germination and seedling growth. For very small seeds, such as poppy, much smaller pots or containers can be used. The pots are two-thirds filled (about 4 cm below the top of the pot) with garden soil. One hundred seeds are sown on the soil surface of each pot and are covered by a few centimetres of sand. The depth of the covering layer depends on the size of the seeds. Smaller seeds should

be covered with less sand. However, the sandy layer should not be less than 1 cm. For cereals, a 4 cm sand layer is usually applied. Pots are transferred into a greenhouse, growth chamber or light room with a temperature typical for seed germination of the particular crop. Seeds of each treatment combination, including control (parent cultivar), should be sown in three pots, composing three replications. They should be watered according to the normal practice for the crop. All emerged seedlings should be counted in each pot when the number of seedlings in the control treatment is stable and no longer increasing. The average number of emerged seedlings from three pots of the control combination is taken as 100 percent of emergence. The reduction of the emergence in treated combinations is calculated relative to the control. For example, when the average emergence in the control is 96.2 seedlings and the average emergence in the lowest dose is 84.6, the emergence reduction is equal to 12.1 percent, according to the formula:

Emergence reduction (percent) = 100 - ((Average emergence in the dose × 100) / Average emergence in the control)

It is useful to present the emergence reduction data in the form of a figure (Figure 8.3).

In cereals, the seedling growth reduction can be measured when the second leaf becomes visible in the majority of seedlings of the control combination. For this purpose, the seedlings are cut on the sand surface and the height of each seedling should be measured with an accuracy ±0.5 cm (Figure 8.4). The calculation of results is similar to the previous one:

Seedling growth reduction (percent) = 100 – ((Average height of seedlings in the dose × 100) / Average height of seedlings in the control)

FIGURE 8.3
Emergence reduction of buckwheat (*Fagopyrum esculentum*) seedlings after seed treatment with 0.75-3.75 mM MNU (3 h, 24^{0}C)

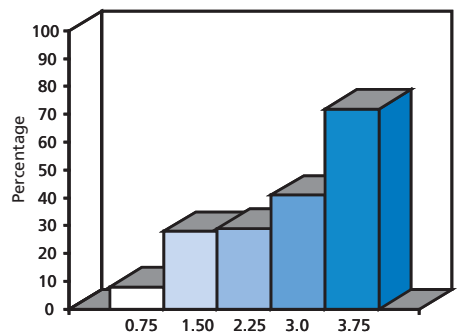
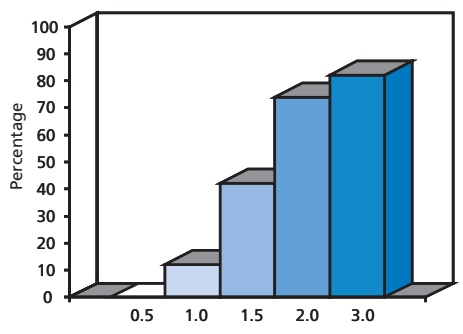


FIGURE 8.4
Seedling growth reduction of barley cultivar 'Maresi' after seed treatment with 0.5-3.0 mM MNU (3 h, 24^{0}C)



In tropical countries, this test can be done in a nursery bed or in hydroponics. The experiment should be organized in three blocks with randomly distributed small plots, each for 100 seeds. For cereal seedling growth reduction, a method called 'blotter sandwich' or 'growing-rack' can also be used. The method was developed by Professor C. Konzak in the early 1960s (FAO/IAEA, 1977). However, it needs a filter paper and special, but simple, equipment.

Pilot experiment

Regardless of the test used, any dose causing a reduction in emergence or seedling growth of greater than 30 percent is considered too high for a large-scale breeding programme with mutants. However, this is rather a general recommendation, as all somatic and genetic effects of the mutagen very much depend on the genetic background of the material treated, with strong differences not only between species but also between cultivars or breeding lines of the same species. For this reason, it is worthwhile to organize a pilot experiment to help compare the somatic and genetic effects induced by a range of doses in one or a few of the genotypes chosen for the breeding programme. The pilot experiment will delay the programme for one season, but it helps in proper selection of the dosages for further experiments with particular genotype(s) and protects the breeder from the failure of a large field experiment.

The best approach is to choose a few genotypes that are promising from the breeder's point of view, and to treat this material with at least four to five different doses of the mutagen, selected on the basis of the results from the pot test. Seeds from each dose and parent should be sown in plots (three replications, randomized blocks) according to normal agronomic practice. It depends on the crop, but usually one hundred seeds per plot should be enough for this purpose. The somatic effects listed earlier can be observed in the M_1 generation. However, it is most important to measure the growth, fertility and survival reduction at maturity. For survival reduction, all plants from the plot should be harvested and counted, except fully sterile plants. Based on the average number of surviving plants in relative to the control, the reduction in survival for each dose is calculated, using the same formula as for emergence reduction, described above. Similarly, the measurement of the plant height of randomly selected 20–30 plants from each plot gives growth reduction. The simplest way to evaluate fertility reduction is to thresh all plants from the plot and weigh all seeds. In most species, these seeds, which are the M_2 generation, can be sown in containers to evaluate the genetic effect of the applied dose of mutagen on the basis of the frequency of point (gene) mutations. In practice, this can be done on the basis of the frequency of chlorophyll mutants among seedlings of the M_2 generation, as suggested by Ake Gustafsson for barley in the early 1940s (e.g. Gustafsson, 1941). From many cereal mutants with chlorophyll defects, three are very easily recognized, as the entire seedling has the same colour. These are the mutants: *albina* (white), *xantha* (yellow) and *viridis* (pale green). It is possible to find a similar type of mutation in other crops, including dicotyledonous species. For example, chlorophyll mutants can be easily observed in tomato M_2 seedlings at the cotyledon stage, before the first leaf develops. The observation of the mutants should be done a few days after emergence, as most of the chlorophyll mutations are lethal. The frequency of mutations (as a percentage) on a seedling basis (M_{sd}) is calculated according to the simple formula:

$$M_{sd} = (\text{number of mutated seedlings } (albina + xantha + viridis) \times 100)/N$$

where N is the number of all M_2 seedlings analysed for a particular dose. An M_{sd} value greater than 3–4 percent should be considered as very high.

The results obtained from the pilot experiment guides selection of the dose for a large field experiment. The frequency of mutations in applied doses should be

the most important criterion, followed by the breeding objective of the programme. It should also be considered that genes controlling other, often useful, characters can mutate with lower frequency than the genes controlling chlorophyll. After choosing the dose, it is very important to calculate the size of the M_1 generation. In this step, the knowledge of survival and fertility reduction for a particular dose is necessary. These parameters help to calculate how many plants will survive after mutagenic treatment and how many seeds will be harvested as a result of the reduction in fertility in surviving plants. As the pilot experiment was performed under the climatic conditions of one season, it should be also considered that the level of somatic effects under the conditions of the next season could be different. For this reason, is very advisable, for the large-scale experiment, to grow the M_1 generation not only with the selected dose, but also with slightly lower and higher doses to be sure that even under different climatic conditions it will be possible to collect enough mutated seeds for the desired size of the M_2 generation. It is also a good practice to have a small plot of the parent cultivar in the close neighborhood to have a control comparator for the reaction of plants to growing conditions and management.

8.4.2 Handling mutated generations and mutant selection

M_1 generation

Mutagen-treated seeds should be sown in fertile soil and grown under good management practices, including the use of fertilizers. It is very important to maintain the M_1 crop at the proper soil moisture level, as plants with somatic effects are much more sensitive to stresses, especially drought. The use of herbicides should be avoided and replaced by mechanical means as some active components of herbicides, often also mutagenic, can influence the growth and development of injured plants. Sowing at double spacing in rows is often applied to avoid competition between M_1 plants with different levels of somatic effects. Good tillering will also allow exploitation of all mutations from different initial cells. When a high dose of mutagen is used, a significant delay in maturity should be expected.

As a multicellular tissue was the subject of mutagenic treatment and a different spectrum of mutations was induced in each cell, the tissues developing from the embryo carry cells with differently modified genomes and are chimeric. Induced genetic polymorphism among initial cells of the sporogenic layer influences the segregation ratio in the M_2 generation. The mutations in cells of somatic tissues are not transferred to the next sexual generation. Some morphological mutations in somatic tissues, such as chlorophyll defects, are often visible and they clearly illustrate the chimeric structure of the M_1 plant (Figure 8.5). The appearance of chlorophyll defects is a good indicator of genetic action of the mutagen. Somatic tissue chimeras are a valuable source of genetic variation in breeding of vegetatively propagated crops.

The method of harvesting M_1 is a key issue in exploiting induced genetic variation in sexually propagated crops. The method applied reflects various factors, including the biology of reproduction of a particular crop species, the cost of labour and of the selection method, the objective of breeding, and the possible size of the M_2 generation. Different methods can be chosen, ranging from the collection of only one seed per plant, to bulk harvesting of entire M_1 plots for development of the M_2 population. From a theoretical point of view, the best

FIGURE 8.5
Chlorophyll defects in M_1 generation after MNU treatment

Notes: a) xantha sector in barley; b) albina and xantha sectors in maize; c) large albina sector in narrow lupine; d) xantha sector in faba bean; e) xantha sector in pea; f) albina, xantha and viridis sectors in backwheat.

way is to harvest separately and thresh the spikes, pods or fruits from each individual M_1 plant and sow seeds using a spike-, pod- or fruit-to-row method. However, this is a rather unrealistic approach in practice. More often, in breeding programmes, a few spikes, pods or fruits from each M_1 plant are harvested, threshed and sown together.

M_2, M_3 and M_4 generations

Normal agronomic practices should be applied for cultivation of the M_2 and M_3 generations. Double-spaced sowing or planting can be used if changes in some morphological characters are the subject of selection in the M_2 generation. However, it should be considered that the small size of the sector built by a single initial cell and the high level of sterility in spike, pod or fruit of the M_1 plant can lead to few recessive forms in the segregating M_2 generation. Numerous mutations are not recognized in the M_2 generation, being obscured by the heterozygous stage. However, they will give Mendelian segregation in the M_3 generation. For this reason, it is good practice to postpone selection to the M_3 generation. Additionally, selection in the M_3 is done on a row or plot basis of homozygous plants, which significantly helps in distinguishing plants with only small, but often agronomically very important, morphological and especially quality characters. This is also the best way to evaluate resistance to biotic and abiotic stresses.

Depending on the generation in which the selection was performed, the homozygosity test can be done in M_3 or M_4. The preliminary evaluation of agronomic traits can be done with selected mutant lines in the M_4 generation. Crosses with a parent variety, other mutants or promising breeding lines are also often initiated in the M_4 generation.

Advanced generations

The selected mutants along with the parent cultivar should be entered into national yield, disease and pest nurseries wherever possible. Growing the mutants in multilocation trials and as off-season crops helps in advancing the generations, while evaluating performance in agroclimatic environments different from that of the main experimental site helps in selecting mutants with wider adaptability. The breeders should also ensure availability of adequate quantity of seed to enter in the regional or national trials. It is also desirable to use these homozygous mutants in the conventional cross-breeding programme of the station. Information on official release of new cultivars derived through mutation techniques should be sent to the MVD at the Joint FAO/IAEA Division, Vienna, Austria. Such information can help other breeders to determine appropriate dosages or selection procedures for developing new, improved cultivars.

8.4.3 Induced mutations in doubled-haploid systems

Doubled-haploid (DH) techniques, such as anther and microspore cultures, wide hybridization, and ovary and ovule cultures have become well established in a range of economically important crop species, including major cereals and the brassicas (Maluszynski *et al.*, 2003). Application of DH system in a conventional breeding programme saves many generations for the production of pure breeding lines. It also enhances effectiveness of selection of desired recombinants, especially when quantitative traits are evaluated.

The same benefits are evident when a DH system is employed in the process of mutant induction and selection. The most important advantages of applying DH systems in mutagenesis include the shortening of time needed for selection of true-to-type mutants; immediate fixation of mutated genotypes in the homozygous stage; screening for recessive mutations in the first generation after mutagenic treatment; and avoiding chimeric structure in M_1 plants. Additionally, if mutant selection

can be carried out *in vitro*, the haploid cells or embryos provide an extremely large mutagenized population, increasing the probability of identifying a rare mutation event. The advantages of combining mutation techniques with DH systems are apparent only when an efficient procedure of DH production is available for a particular crop. The recent progress in developing effective protocols for DH production, especially through isolated microspore culture, has made possible the application of mass-scale *in vitro* mutagenesis and selection methods in major cereals: wheat, barley and maize, similar to the previous achievements in oilseed rape. However, DH protocols are difficult to directly transfer between laboratories, and the success in producing a sufficient number of DH plants depends very much on the breeder's ability to grow high quality donor plants.

There are two main approaches for the use of haploid systems for mutant production. Most often, mutagenic treatment is applied to haploid cells (microspores) or organs containing haploid cells (anthers, spikes, panicles or flower buds) at, or before, *in vitro* culture. Mutagenic treatment of isolated microspores with gamma, X-ray or UV radiation proved to be an efficient method for mutation induction in oilseed rape. Chemical mutagens can also be applied to haploid cells *in vitro*, but the mutagenized cultures are more difficult to handle because of the requirement for extensive washing in order to remove the mutagen residues. The application of mutagenic treatment to the haploid cells or tissues in *in vitro* culture usually drastically decreases their regeneration ability. For this reason it is advisable to perform a mutagen sensitivity test for the haploid cell or embryo survival and regeneration capacity before setting up a large-scale experiment. It should be noted that the doses of mutagens applied to cells *in vitro* should be at least an order of magnitude lower than the doses used for seed treatment. In *Brassica napus*, the irradiation doses used for mutation induction in isolated microspore cultures ranged from 5 to 15 Gy for gamma rays, 10 to 40 Gy for X-rays, and a dose rate of 33 erg mm^{-2} s^{-1} for 10 to 60 s with UV rays. In microspore cultures of barley treated with sodium azide, the mutagenic treatment lasted only 1 hour, and the concentration of the applied mutagen has not exceeded 1^{-4} M.

Another approach to mutagenic treatment with the use of a DH system relies on using M_1 plants derived from mutagenized seeds as donors for haploid production. In this method, seeds are treated with the doses of physical or chemical mutagens used in conventional seed mutagenesis. Treatment of dormant seeds instead of haploid cells in culture allows for application of much higher doses of mutagens, which provide the higher frequency of mutations in the DH population. Avoiding the somatic effects of mutagenic treatment on *in vitro* regeneration ability is another advantage of this procedure in comparison with the treatment of microspores in culture. Use of M_1 plants as donors for anther culture has been successfully demonstrated in barley and rice. In Peruvian barley cultivars, numerous DH mutants were produced from anther culture of M_1 plants developed by treatment with MNU and sodium azide. In rice, anther culture of gamma-irradiated M_1 plants resulted in the development of a short-duration upland rice mutant line, which in Myanmar matured 19 days earlier than the parent variety.

The mutagenic treatment of haploid cells can be followed by selection applied at the *in vitro* stage. If the selected trait is

expressed equally at the haploid cell or embryo and the plant levels, it is possible to apply a selection factor at the *in vitro* stage, maximizing the population size of individuals (cells or embryos) screened for a particular mutation. The selective agent, e.g. herbicide, should be used at a concentration near to LD_{100}. The feasibility of this system has been verified by recovering herbicide- and disease-resistant mutants in oilseed rape after *in vitro* mutagenesis and selection applied to isolated microspore cultures.

Oilseed rape haploid or DH embryos provide an excellent target for another early selection technique. Microspore-derived and zygotic embryos proved to have almost identical fatty acids composition and glucosinolate content. This was used to identify haploid embryos with the desired fatty acid composition, based on the analysis of one cotyledon. This non-destructive method of analysis allowed isolation of homozygous oilseed rape mutants with increased level of oleic acid and accompanying reduction of linoleic acid.

8.5. INDUCED MUTATIONS IN MOLECULAR BREEDING – TILLING

Recent advances in plant genomics, especially large-scale genome sequencing, have opened new possibilities for application of mutation techniques in crop improvement. Using the reverse genetic strategy called TILLING (Targeting Induced Local Lesions In Genomes), it is possible to induce a series of alleles in a target locus, providing that its sequence is known (McCallum *et al.*, 2000). The TILLING strategy was initially developed for model plant and animal species as a discovery platform for functional genomics, but soon it became a valuable tool in crop breeding as an alternative to the transgenic approach. The TILLING technique relies on a high frequency of mutations induced by chemical mutagenesis, combined with a high-throughput screening method for single-nucleotide polymorphisms (SNPs) in the targeted sequence. The feasibility of this technology for generating a series of new alleles in a gene of interest has been already demonstrated in barley, maize and wheat, not to mention model organisms such as *Arabidopsis thaliana*, fruit fly, zebrafish and rat. Identification of 246 alleles of the *waxy* gene among EMS-treated M_2 individuals of bread and durum wheat is the best example of the potential of TILLING in creating new alleles of a gene responsible for an economically important character.

The basic TILLING methodology has the following steps:

- creation of a mutated population (M_2);
- isolation of the DNA from M_2 plants;
- PCR amplification of the targeted DNA segment using pooled DNA from M_2 plants as a template;
- denaturation and re-annealing of PCR products to form heteroduplexes between mutated and wild-type DNA strands;
- detection of mismatches in the heteroduplex using different procedures, e.g. cleavage by the specific endonuclease or denaturating high performance liquid chromatography (DHPLC); and
- sequencing the targeted DNA region in M_2 individuals composing the positive pool, for detection of the mutant.

As the first step in the TILLING procedure, large-scale mutated populations are generated. Most often, the chemical mutagen EMS is used for mutation induction, although sodium azide and MNU has also been used in barley and rice. Both these mutagens are known to induce a high frequency of point mutations. Usually, M_1 populations of 10 000–20 000 individuals are grown under good conditions after treat-

ment with two or three doses of mutagen. M_1 plants are harvested individually. Taking into consideration the chimeric structure of M_1 plants, seeds from different spikes, panicles or pods are often threshed separately.

Screening for mutations is performed in the M_2 population. Depending on the programme objective, M_2 populations consisting of several hundreds to 20 000 individuals are created. To prevent redundancy, usually one M_2 plant from each selfed M_1 individual or from one M_1 spike, panicle or pod is sampled. DNA from each M_2 seedling is isolated separately and the M_2 plant is grown to maturity. Each M_2 plant is harvested individually and M_3 seeds are carefully stored.

Screening for mutations in a target gene is based on detecting heteroduplexes between the wild-type and mutant-DNA fragments in the pooled DNA samples. In the first step, polymerase chain reaction (PCR) amplification of the targeted genome region is performed using DNA pooled from 5 to 8 M_2 individuals as a template. Next, the amplified DNA fragment is denatured and re-annealed, which allows formation of heteroduplexes between the wild-type and mutant DNA strands. Many approaches for detecting the mismatched sites within the heteroduplexes have been tried, such as denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), denaturating high-performance liquid chromatography (DHPLC) or cleavage by the specific endonuclease. DHPLC was the first high throughput technology applied for the detection of heteroduplexes with the mismatched sites. However, cleavage of the mismatches with *Cel*I endonuclease (the novel plant enzyme isolated from celery), followed by the analysis of DNA fragments on denaturating polyacrylamide gels has now become the most popular system for mutation detection. The advantage of this procedure is that it eliminates the number of false-positive mutation identifications, as both cleaved DNA fragments are labelled with different fluorescent dyes. With two-colour imaging, a true mutation has two mutant bands below the wild-type band in the same lane. The sum of the length of the mutant bands in a lane must equal the length of the wild-type band in order for the mutation to be confirmed.

Once the mutation is detected in a DNA pool, the target gene region in all M_2 individuals comprising this pool is sequenced and the M_2 plant carrying the mutation is identified. M_3 progeny of the identified mutant are then used for phenotypic evaluation of the mutated trait.

8.5.1 Case study: Development of waxy mutants in bread and durum wheat (Slade *et al.*, 2005)

Development of cereal varieties with waxy starch, composed almost entirely of amylopectine with little or no amylose, has been one of the most important objectives of commercial plant breeding. Waxy starch wheat has broad potential commercial uses in the food, paper and adhesive industries for making better quality products. Despite many breeding efforts over the last two decades, there are no wheat varieties with fully waxy starch. Using the TILLING approach for mutation generation and discovery, the research team from Anavah Inc., USA, induced and identified 246 mutated *waxy* alleles in two elite wheat varieties through only one experiment.

Development of TILLING libraries

Seeds of bread wheat (*Triticum aestivum*) and durum wheat (*T. turgidum* subsp. *durum*) were treated with two doses of

EMS. Elite commercial cultivars were used as the genetic material for mutation induction. For the bread wheat cv. Express, 0.75 percent and 1.2 percent EMS treatment was applied, while for the durum cv. Kronos the EMS concentrations were 0.75 percent and 1 percent. Each treatment lasted 18 hours and was preceded by a short (4 minutes) vacuum infiltration (pre-soaking) with H_2O. After treatment, M_1 seeds were washed for 4 to 8 hours and sown to produce M_1 plants, which were allowed to self-pollinate and were harvested individually. TILLING populations of about 10 000 M_2 individuals of hexaploid wheat and about 8 000 M_2 plants of durum wheat were created. To prevent redundancy, only a single M_2 progeny from each M_1 selfed plant was used in the study.

Screening for mutations at the waxy loci

The target sequences in the bread wheat involved 2 114 kb at the *Wx-A1* locus and 1 345 kb of the *Wx-D1* locus. In the durum wheat, 1 232 kb sequence of the *Wx-A1* and 487 kb of the *Wx-B1* genes were screened. DNA from 1 152 bread wheat and 768 durum wheat M_2 seedlings was used for mutation screening. Most of bread wheat individuals (768 M_2) and all M_2 durum plants analysed came from treatment with 0.75 percent EMS. DNA isolated from individual M_2 plants was pooled two to sixfold. The PCR amplification was carried out with the use of specific primers labelled with fluorescent dyes IRD700 and IRD800. The PCR products were digested with the *Cel*I enzyme, denatured and re-annealed. The samples were then separated on denaturating polyacrylamide gel using the LI-COR² DNA sequencer. Images were analysed visually for the presence of the cleaved DNA fragments indicating a mutation in the target region.

Allelic series of mutations in the target loci

In total, 246 new alleles at three *waxy* loci were identified in a population of 1 920 M_2 individuals used in the survey: 196 alleles in hexaploid and 50 in tetraploid wheat. The majority of mutations were G to A, or C to T transitions. Among the identified changes there were 84 missense, 3 truncation and 5 splice junction mutations. These new alleles encode waxy enzymes ranging in their activity from near wild-type to almost zero. A null mutant containing mutations in all three *waxy* homologues, highly desirable for wheat starch improvement, was also isolated. As the authors (Slade *et al.*, 2005) pointed out, the series of alleles created through TILLING in one experiment represent more genetic diversity than had been described in the preceding 25 years.

8.6. MUTATION TECHNIQUES FOR THE IMPROVEMENT OF MAJOR CROPS

8.6.1 Induced mutations in cereals

Cereals, of all crop species, have most often been the subject of improvement through the use of mutation techniques. In addition to major cereals such as rice, barley, wheat and maize, other species, including some exotic ones, have also been the subject of mutagenic treatment in several countries (Table 8.2). The majority of more than 1 000 mutant cultivars were obtained after radiation, especially gamma ray treatment, and directly released. However, the ease of mutagenic treatment rather than the kind of mutagenic specificity determined the use of radiation rather than chemical mutagens. Additionally, the bio-hazardous nature of most mutagenic components prevents their use in simply equipped laboratories. Nevertheless, numerous valuable mutants have been obtained with the use of MNU, EMS and sodium azide. Another tendency, observed more recently, is to transfer the

TABLE 8.2
Species and the number of officially released cereal mutant cultivars (FAO/IAEA Mutant Varieties Database – actualized)

Crop species	Common name	No. of released mutant cultivars			No. of countries with released mutant cultivars
		Total	Direct	Cross	
Avena sativa	Oat	21	5	16	4
Coix lachrymal-jobi	Job's tears	1	1		1
Fagopyrum esculenthum; F. sagittatum	Buckwheat	8	6	2	1
Hordeum vulgare	Barley	269	53	216	28
Oryza sativa	Rice	434	291	143	31
Panicum spp.; *Setaria* spp.	Millet	25	22	3	4
Pennisetum spp.	Pearl millet	5	3	2	1
Psathyrostachys juncea	Russian wildrye	1	1		1
Secale cereale	Rye	4	4		2
Sorghum bicolor	Sorghum	13	12	1	4
Sorghum durra	Durra	1	1		1
Triticum aestivum	Wheat	197	148	49	18
Triticum turgidum subsp. *durum*	Durum wheat	30	10	20	5
Zea mays	Maize	68	12	56	6

mutated gene to other cultivars by crosses. This is especially evident in barley, where 216 out of 269 mutant cultivars were developed by introduction of a mutated genetic source, mainly genes for semidwarfness, into new genetic backgrounds, and in maize, where only 12 out of 68 mutant cultivars were directly released.

Mutagenic treatment

Seed treatment is most often applied for mutagenesis of cereals. The pollen treatment method was developed by the team of Professor M.G. Neuffer from the University of Missouri, Columbia, USA, and successfully applied in maize to establish the largest mutant collection widely utilized in breeding and basic research (Bird and Neuffer, 1987). More recently, due to the success in DH production, microspore or anther cultures have become the subject of mutagenic treatment in major cereals.

All steps described in Section 8.3, 'Mutagenic treatment', should be followed in the treatment of seeds. For major cereals, 8 to 10 hours of pre-soaking in water are usually applied before treatment with chemical mutagen. To facilitate the work of plant breeders starting a mutation breeding programme, the doses of physical and chemical mutagens employed for seed treatment in some cereal species are listed in Table 8.3. These data can be helpful when planning experiments to estimate the critical dose and for the subsequent pilot programme or large-scale experiment.

Growing and handling the early generations

Growing the M_1 generation on fertile soil free of biotic and abiotic stresses is important for the production of adequate seeds for the M_2 generation. In rice, M_1 seeds are sown in the nursery, and seedlings are transplanted to the field according to the local practice. In cross-pollinated species, the M_1 plants should be kept isolated from untreated material. To grow plants under good conditions is a relatively easy task as the area needed for the M_1 population is small,

TABLE 8.3
Doses of physical and chemical mutagens used for seed treatment in cereals

Crop species	Mutagen	Range of doses
Rice	Fast neutrons	3–8 Gy
	X-rays	95–250 Gy
	Gamma rays	100–350 Gy
	MNH (MNU)	(0.7–1.5 mM) × (3–5 h)
	ENH (ENU)	(1.7–2.5 mM) × (3–5 h)
	EMS	(0.2–0.5 percent) × (8–20 h)
	NaN_3	(0.5–2 mM) × (3–5 h)
	EI	(0.01–0.03) × (3–6 h)
	DMS	(0.01–0.05 percent) × (4–6 h)
	Streptomycin	(800–2400 ppm at 15°C) × 40 h
Wheat	Fast neutrons	2–6 Gy
	Thermal neutrons	1×10^{11} N/cm^2 – 8×10^{12} N/cm^2
	X-rays	150–250 Gy
	Gamma rays	50–350 Gy
	MNH (MNU)	(0.75–1.5 mM) × 5 h
	EMS (ENU)	(0.01–0.04 percent) × (10–30 h)
	NaN_3	(0.5–2.0 mM) × 5 h
	DMS	(0.005–0.04 percent) × 5 h
	DES	(0.4–1.0 percent) × 5 h
	EI	(0.04–0.09 percent) × (3–5 h)
	Streptomycin	(0.1–0.2 mg/ml) × (12–48 h)
Barley	Fast neutrons	2–5 Gy
	Thermal neutrons	4.0×10^7 N/cm^2 – 6.5×10^7 N/cm^2
	X-rays	60–200 Gy
	Gamma rays	150–400 Gy
	MNH (MNU)	(0.5–1.0 mM) × 5 h
	ENH (ENU)	(1.0–2.5 mM) × 5 h
	EMS	(0.02–2.5 percent) × (8–20 h)
	NaN_3	(0.5–1.5) × 5 h
	Ethylene oxide	(0.02–0.04 percent) × (15–20 h)
	DMS	(1.0–1.5 percent) × (8–12 h)
	EI	(0.03–0.06) × (8–12 h)
Millet	Gamma rays	200–400 Gy
	DMS	(0.02–0.05 percent) × (15–20 h)
	MNH (MNU)	(1.0–1.7 mM) × (3–5 h)
	Streptomycin	(800–2 400 ppm at 15°C) × 40 h
Sorghum	Gamma rays	150–300 Gy
	NaN_3	(1.0–4.0 mM) × 4 h

Notes: The chemical mutagenesis was usually applied at a temperature of 20–24°C.

even if three doses of mutagen have been applied. Treatment of 2 000–4 000 seeds per dose should be sufficient to obtain a large M_2/M_3 generation. However, the size of the M_2 depends on the method of seed collection from the M_1 generation. If M_1 has been set up in three doses, the breeder can choose a population with a significant number of seeds during the harvest. They should consider the level of somatic effects, such as mature plant growth reduction, and fertility and survival reduction. The final decision on which dose seeds should be chosen for the M_2 generation can be taken based on the genetic effect of the mutagen. As M_1 plants are usually harvested individually (pulled with roots), the spikes or panicles can be collected from a few hundred plants and planted, without threshing, in boxes for evaluation of the frequency of chlorophyll mutants. Depending on the objective of breeding, and considering somatic and genetic parameters of mutagenic treatment, the breeder can choose from which dose to take the seeds for the M_2 generation. Plants from the other two doses can be threshed and seed stored as a reserve of treated material.

In classical work, one or a few spikes or panicles from each individual M_1 plant are treated separately: collected, threshed and sown as plant progenies, head-to-row method. Various modifications of this approach have been applied, such as harvesting single heads from each M_1 plant in bulk and sowing seeds in rows after threshing. Should these approaches be too laborious, mechanical harvesting in bulk, threshing and sowing can be also used. If selection is initiated in the M_2 generation, i.e. on a single-plant basis, seeds are usually sown double spaced. However, taking into consideration the low occurrence of recessives in the M_2 generation, it is strongly suggested to postpone selection to the M_3 generation, which will allow selection to be carried out on a progeny basis. This is especially important if selection for biotic and abiotic stress tolerance or resistance is done under field conditions in the stress-prone area. In this approach, as many as possible, randomly chosen, M_2 plants are harvested, threshed and sown on a plant-progeny basis. This method is more laborious but increases the probability of selecting numerous plants homozygous for a desired character and helps avoid selecting false-positive mutants. To avoid having to thresh individual heads, the most laborious part of this method, interesting modifications were successfully used for the selection of semi-dwarf barley and salt-tolerant rice. In both cases, heads from each M_2 plant were harvested and sown—without threshing—the next season directly in the field. In the case of rice, the panicles were sown in a saline area. Numerous surviving lines, with mutants homozygous for these characters, could be directly selected, in addition to segregating progenies. Several hundred thousand M_2 plants could be characterized in the M_3 progenies using this approach.

Generally, both M_2 and M_3 generation are grown using normal agronomic practices. Any modification in plant cultivation depends mainly on the selection method. If the selection of desired mutants was initiated in the M_2, the seeds from selected plant (M_3) are sown on a progeny basis to check mutant homozygosity.

8.6.2 Case study: Development of malting barley cultivar 'Diamant' (Source: Based on FAO/IAEA, 1977)

The variety 'Valicky' was chosen for mutagenic treatment to improve lodging resistance and yield. This cultivar with high malting quality was first released (under the

name 'Valticky pivovarsky') in Moravia in the early 1920s, as a landrace selection of a shorter form of local cultivar 'Proscovcuv hanacky'. Valticky was re-released after the Second World War as a synthetic population of two types of this cultivar called types A and B, at this time grown in Moravia.

1956 6 000 dry seeds of cv. Valticky irradiated with 100 Gy of X-rays. The M_1 plants harvested individually.

1957 M_2 grown as plant progenies. Selection initiated in M_2. In one progeny, No. 228, higher tillering, short-straw mutants were detected.

1958–61 Progeny testing and seed increase of previously selected mutant line, designated as VR_Z.

1962–64 State variety trials including line VR_Z at multiple locations, demonstrating 10 percent higher average yields than other cultivars in the 31 trials.

1965 Official registration of new cultivar 'Diamant' in Czechoslovakia, differing from the parent cv. Valticky by the following characters: culm 10–15 cm shorter due to shorter internodes; about 10 percent higher yield, but equal quality of grain and malt; number of fertile tillers increased by 2–3; and tillering delayed by 10–14 days. Genetic investigation indicated that mutation at *denso* (*sdw1*) locus, mapped to chromosome 3 (3H), was responsible for the changed characters.

8.6.3 Induced mutations in legumes

Faba bean (*Vicia faba* L.) was extensively used in the early period of plant mutagenesis for investigating the effect of ionizing radiation and chemical mutagens on chromosomes. 'N.C. 4-X' was the first groundnut cultivar released for cultivation in the United States of America in 1959. It was developed by Gregory at North Carolina after exposing seeds to X-rays. Since then, several mutant breeding derived cultivars of legume crops have been released for commercial cultivation, increasing from 265 in 1999 to 337 to date (MVD, no date). The most common plant characters altered in the new cultivars are listed in Table 8.4. Theoretically, it should be possible to obtain mutation at any of the 25–30 000 loci currently estimated for plants, provided there are means to identify the induced changes, and can be used for screening large populations. Most of the mutation experiments are limited to the identification and selection of the 'visible' mutants in the field. These include mutations affecting the characteristics listed in Table 8.4. In addition, a large number of mutants altering symbiosis with nitrogen fixing micro-organisms have been identified in legumes using appropriate screening methods. Such mutants have been isolated in pea, soybean, common bean, faba bean, chickpea, groundnut and pigeon pea, and include mutants that either do not produce, have few or have ineffective root nodules. Hypernodulating and mutants that produce nodules even at otherwise inhibitory levels of nitrate concentration have been isolated after mutagenizing seeds in pea, soybean and common bean (Bhatia, Nichterlein and Maluszynski, 2001). A new soybean cultivar 'Nitrobean 60' that gave higher yield and contributed a greater amount of fixed N to the following cereal crop was developed in Australia after crossing an induced hypernodulating mutant. A day-length insensitive mutant in *Sesbania rostarata,* a green manure plant

TABLE 8.4
Most frequent and other characters altered in legume crops

Character most frequently modified	Yield, plant type, erect habit, dwarfness, branching habit, phytomass (biological) yield, leaf size and shape, flowering time, maturity, flower colour, number of flowers, pod and fruit characters (size, length, number of seeds per pod or fruit, non-shattering pods), seed and kernel size, seed coat colour
Other characters modified	Day length insensitivity in pigeon pea, mungbean and sesbania
	Afila-type mutant in pea resulting in modification of leaflets into tendrils which facilitates mechanical harvesting of green peas
	Terminal inflorescence in pigeon pea and faba bean
	Higher shelling percentage, thick or thin pod cover, harvest index, seed dormancy in groundnut
	Cotyledon colour in dry seeds
	Cold and drought tolerance in soybean and pea
	Resistance to bacterial, fungal, and viral diseases in several crops, and insect resistance in some
	Lodging resistance
	Drought resistance
	Superior nutritive value and protein content in cowpea and pea
	Fodder quality in lupin
	Nodulation mutants with hypernodulation, nitrate-tolerant nodulation, no nodulation and ineffective nodules have been isolated in specially designed experiments

TABLE 8.5
Successful gamma or X-ray doses for dry seed exposure in legume crops[1]

Crop species	Common name	Successful dose range (Gy)
Arachis hypogaea	Groundnut	150–250 (11)
Cajanus cajan	Pigeon pea	160 (1)
Cicer arietinum	Chickpea	100–200 (5)
Dolichos lablab	Hyacinth bean	240 (1)
Glycine max	Soybean	100–200 (20)
Phaseolus vulgaris	Common bean	100–200 (5)
Pisum sativum	Pea	100–200 (5)
Vigna radiata	Mungbean	100–200 (7)

Notes: (1) 'Successful doses' defined as the doses that led to the development, registration and release of a mutant cultivar directly without using the mutant as a parent in crossbreeding. The number of released cultivars is in brackets.

that produces aerial nodules on the stem, was identified when the crop was grown in the off season. The mutant produces tall plants with large phytomass and N-fixing nodules irrespective of planting time.

Mutagenic treatment

Gamma ray or X-ray exposure of the dry seeds is the most convenient method for creating genetic variability in legume species. Successful dose ranges—defined as the dose that led to the development, registration and release of mutant cultivars directly without resorting to cross breeding—are given in Table 8.5. Exposures of seeds to 100–200 Gy, except for faba bean, resulted in 49 out of 111 legume cultivars developed as direct mutants. Chlorophyll mutated sectors appearing on the first true leaves after seed germination in leguminous plants can be used to monitor the effect of radiation and chemical mutagens.

TABLE 8.6
Method for isolation and induction of mutations for use in breeding of grain legume crops

Generation	Operations
M_1	Expose 5000 to 10 000 seeds of the best available cultivar to 100–200 Gy of gamma or X-rays. Plant the M_1 generation following normal cultivation practices.[1] Harvest the first five pods, or all the pods, from each of the M_1 plants.
M_2	Grow the M_2 population as plant-progeny rows (minimum about 50 000 M_2 plants). Look for all morphological and physiological changes in each M_2 plant from seedling stage to harvest, and harvest the selected plants individually.
M_3	Grow the selected mutants as single-plant progenies and check for the segregation of the desired trait. Continue selection for the desired trait on single-plant basis. Uniform, non segregating mutant progenies, if any, can be bulked at this stage to hasten the breeding cycle.
M_4	Evaluate the expression of the selected trait and yield of the bulk lines in comparison with the parent as well as with the best check cultivar. Record observations on all agronomic parameters, disease and insect resistance in comparison with the parent cultivar. Repeat the procedure for the single-plant selections as outlined above.
M_5 onwards	Follow normal plant breeding procedures with the selected progenies. Evaluate the selected lines at more than one location. Enter one or two of the best lines at a time in national and regional evaluation trials, or to local farmers in participatory breeding programmes. Initiate seed multiplication to meet the demand of the mandatory trials for official approval and release of the cultivar.

Notes: (1) Outcrossing is increased in the M_1 plants due to pollen sterility induced by mutagenic treatments. It is desirable to grow the M_1 plants in isolation for facultative crosspollinating species. Selfing of the M_1 plants is essential for genetic experiments.

Growing and handling of the early generations

The general procedures for growing and handling the early generations are outlined in Table 8.6. These are based on over forty years of mutation experiments with groundnut, black gram, mung bean, pigeon pea, soybean and *Sesbania* sp. at the Bhabha Atomic Research Centre, Bombay, India, where over twenty new cultivars have been released for cultivation that have been developed using induced mutations. The procedures shown in Table 8.6 were used for the selection of early flowering, plant type, pod and seed size, and for other yield component traits. It was observed that it is relatively easy to find mutants for one of the yield components, such as number of pods per plant, pod size and length, number of seeds per pod or seed weight. Such mutants may not be superior to the parent in yield *per se*. Hybridization between mutants individually superior in yield components resulted in selection of genotypes significantly higher in grain yield over the parent cultivar.

8.6.4 Case study: Development of black gram (*Vigna mungo*) cultivar 'TAU-1' (Information provided by Drs R.G. Thakare and S.E. Pawar)

'No. 55' was the best prevailing cultivar in the state of Maharashtra, India, with the drawback of small seed size (low hundred-seed weight of about 4 g). Constraint analysis indicated that increase in seed weight might enhance yield.

1974 500 seeds each exposed to 100, 200, 300, 400 and 500 Gy gamma rays. M_1 was grown and harvested as single plants.

1975 Single M_1 plant progenies grown as M_2 population. Approximately 400 progenies, 4 radiation doses and 30 plants per progeny, totalling to 48 000 plants, were screened for

mutants with alteration in plant type, yield components and seed size. Three large seed size and other putative mutants identified. Large-seed mutants were obtained following 300 Gy exposure.

1976 All mutants were progeny tested, and further selection of single plants continued in the M_3 and M_4 generations. True breeding lines were isolated, three with large seed size and 65 for other characters.

1977-78 Large seed size mutants evaluated in yield trials with parent No. 55 and a newly approved cultivar 'T-9'. Large seed size mutant yields were superior to the parent No. 55, but less than T-9 with still smaller seed size (hundred seed weight <4 g).

1979 Large seed size mutant lines 'UM-196' and 'UM-201' hybridized to T-9.

1980-81 F_1 and F_2 populations were grown, and selections made for large seed size, T-9 plant type and yield components, and advanced to F_3 and F_4.

1982-84 Selection '80-7' was found to give the highest yield in on-station trials.

1985-87 Line 80-7 was entered in the evaluation trials of the Punjabrao Agricultural University, and subsequently in the trials of the Coordinated Pulses Improvement Programme of the Indian Council of Agricultural Research. 80-7 gave 24 percent and higher yield over No. 55, and 9 percent over T-9, the national check cultivar. Its mean hundred seed weight was 4.8 to 5.0 g. It was first released for the Vidarbha region of Maharashtra State, India, in 1987, and later for the entire state, and also for Karnataka State in India.

At the time of official release as TAU-1, 150 kg of Breeder's, 3 560 kg of Foundation and 1 440 kg of Certified seed were available. Foundation seed production reached 40 tonne during 1994–95. Two crops per year were grown for experimental work.

8.6.5 Induced mutations in oil and fibre crops

Mutation techniques have been used for improvement of annual oil crops and crops providing bast and seed fibres (Table 8.7). Breeding objectives for using induced mutations are similar to other crops, although for oil crops there are unique objectives for the modification of oil quality (Table 8.8). Most of the oilseed mutant-derived cultivars released have been developed directly from mutants, but 50 percent of groundnut and 67 percent of linseed mutation-derived varieties were developed from

TABLE 8.7
Oil crops and fibre plants improved through induced mutations

Latin name	Common name
Oil crops	
Arachis hypogaea	Groundnut
Brassica campestris	Turnip rape
Brassica juncea	Indian mustard
Brassica napus	Rapeseed
Euphorbia fulgens	Euphorbia
Glycine max	Soybean
Helianthus annuus	Sunflower
Linum usitatissimum	Linseed
Ricinus communis	Castor bean
Papaver somniferum	Opium poppy
Sesamum indicum	Sesame
Fibre plants	
Boehmeria nivea	Ramie
Corchorus capsulari	Jute
Corchorus olitorius	Tossa jute
Gossypium sp.	Cotton
Linum usitatissimum	Flax

TABLE 8.8
Most frequent and other characters altered with induced mutations in oil and fibre crops

Oil crops	Fibre crops
Characters most frequently modified	
Higher yield	Higher yield
Altered plant type	Plant vigour
Early flowering	Earliness
Early maturity	
Higher oil content	
Other characters modified	
Modified oil quality (such as high oleic, low linolenic acid content)	Disease resistance
Increased resistance or tolerance to diseases and pests	Improved stress tolerance
Resistance to shattering	Lateness
Improved drought tolerance	

crosses with mutants. In fibre crops, 75 percent of the varieties have been directly developed, mainly through treatment with gamma radiation or X-rays, and 25 percent through crosses with mutants. For some of these varieties, in both oilseeds and fibre crops, remarkable economic gains have been reported. In breeding of oilseed rape and sunflower, spontaneous and induced mutants have been used in combination with conventional breeding methods to modify oil composition and increase yield.

Traditional oilseed rape has high erucic acid content in the oil and high glucosinolate levels in the meal, and both components are nutritionally undesirable. They have been reduced by breeders after identifying and using spontaneous mutants in the development of canola with less than 2 percent erucic acid and less than 30 µM/g of aliphatic glucosinolates in the meal. This process was facilitated by the development of analytical methods for quality assessment suitable for screening individual plants or single seeds of large breeding populations. Canola quality has been developed for *Brassica campestris* (turnip rape), *Brassica napus* (oilseed rape) and *Brassica juncea* (Indian mustard). Further improvements were made using crosses with EMS-induced low-linolenic-acid mutants and radiation-induced high-oleic-acid mutants. The expansion of canola cultivation in Canada and Europe is primarily due to its modified fatty acid composition, i.e. low erucic acid, low linolenic acid and high oleic acid, combined with a low content of glucosinolates, which improved the nutritional quality of the oil for human nutrition, processing and storage, and the meal for livestock feed. Microspore mutagenesis has also been used in combination with *in vitro* screening for the development of *B. napus* tolerant to the herbicides imidazoline and chlorosulfuron. In Canada, Australia and Europe, many *Brassica* varieties with a modified oil-profile are based on mutant germplasm (Bhatia, Nichterlein and Maluszynski, 1999).

A number of mutant varieties with changed oil composition have also been developed in other species. In sunflower, after mutagenic treatment with dES, the mutant cultivar 'Pervenets' with high oleic and low linolenic acid content was developed, and widely used for hybrid production with high oleic acid content in the United States of America and Europe, to produce oil with the high oxidative stability preferred for

food processing (e.g. frying). The oil with high oleic acid content has steadily gained market acceptance, leading to increasing cultivation areas of high-oleic sunflower cultivars. In linseed, a doubled mutant has been developed after crossing two individually selected mutants with reduced linolenic acid obtained after EMS treatment, and used in further crosses, resulting in the release of a number of low-linolenic-linseed cultivars in Australia and Canada.

In cotton, the development of 'NIAB-78', a gamma-ray induced, high yielding mutant cultivar, released in 1983, had great economic impact in Pakistan. Developed at the National Institute of Agricultural Botany, NIAB-78 had a marked influence on sustaining the textile industry of Pakistan, and contributed to the national economy in several ways. The variety, ideal for a cotton-wheat rotation, had a shorter stature, determinate growth habit, tolerance to heat and escaped bollworm attack due to its early maturity. Within five years of release, its cultivation doubled cotton production in Pakistan, and even after 14 years of its first release, nearly 25 percent of the area under cotton in Pakistan is planted to this cultivar. The new cultivar 'NIAB Karishma', released in 1996, and derived from a cross of the mutant cultivar NIAB-86 with an American strain 'W 83-29', is early maturing, has improved heat tolerance, high yield potential and has been cultivated on 486 000 ha.

Mutagenic treatment

Both ionizing radiation (gamma rays or X-rays) and chemical treatments have been applied to dry, dormant seeds of oil and fibre crops. The successful dose ranges for radiation, defined as the dose that led to the development, registration and release of varieties derived directly from mutants without using mutant crosses are given in Table 8.9. The doses given should be considered as an orientation, because tolerance to seed irradiation can differ between varieties. The recently released variety 'Abasin-95' was developed after gamma ray treatment of the Canadian variety 'Tower' using a much higher dose, 1 400 Gy, than for the development of most of the other oilseed rape varieties. Chemical mutagens were much less used; however, they led to the development of important commercial

TABLE 8.9
Successful gamma or X-ray doses for dry seed exposure in oil seeds and fibre crops

Crop species	Successful dose range (Gy)
Oil crops	
Arachis hypogaea (groundnut)	150–250 (11)
Brassica juncea (Indian mustard)	700
Brassica napus (rapeseed)	600–800
Glycine max (soybean)	100–200 (20)
Linum usitatissum (linseed)	100 (1)
Papaver somniferum (opium poppy)	50 (1)
Ricinus communis (castor bean)	400 (1)
Sesamum indicum (sesame)	100–200 (5)
Fibre crops	
Corchorus capsularis (jute)	250 (1)
Gossypium spp. (cotton)	200–400 (5)

Notes: Successful doses defined as the doses which led to the development, registration and release of mutant variety directly without using the mutant as a parent in cross breeding. The number of released cultivars is in brackets.

varieties with altered oil composition, e.g. high-oleic-acid sunflower (dMS), low-linolenic oilseed rape (EMS) and low-linolenic linseed, the so-called linola (EMS).

Growing and handling of the early generations

The general procedures for growing and handling the early generations of oil and fibre crops are outlined in Table 8.10. For the detection of plants with altered oil composition, the identification of mutants can be done using half-seed methods, screening M_2 seeds harvested from M_1 plants. For traits such as tolerance to biotic and abiotic stresses, it is recommended to do the mutant screening not on a single-plant level in the M_2 but in M_2 progeny rows (M_3). Mutants (M_4) with low agronomic performance, but valuable mutant traits, can be improved through backcrossing to the parent or by other crosses.

TABLE 8.10
Method for isolation and induction of mutations for use in breeding of oil and fibre crops

Generation	Operations
M_1	Expose 5 000 to 10 000 seeds of the best available variety or homozygote line to gamma or X-rays
	Plant the M_1 generation under optimal conditions to produce M_2 seeds, following normal cultivation practices, growing them in isolation or bagging before flowering.
	Harvest the first two to five pods, capsules, bolls or heads; the M_1 plants can be harvested individually if the M_2 will be grown in progeny rows, or in bulk if the M_2 will be grown in bulk.
M_2	For traits expressed and visible at the single-plant level: grow the M_2 population as plant-progeny rows (30–50 plants from each M_1 plant), and remaining seed can be stored for sowing in subsequent seasons.
	Look for all morphological and physiological changes in each M_2 plant from the seedling stage to harvest, and harvest the selected plants individually.
	For traits expressed at the seed level (e.g. oil composition in oil seeds): cut part of the M_2 seed (linseed, sunflower) or germinate M_2 seed (*Brassica* spp.) and cut one cotyledon for fatty acid analysis, then continue cultivating the seed or seedlings with desired fatty acid composition.
	For traits not expressed at the single-plant level: multiply seed of each M_2 plant, and harvest single plants for row evaluation in M_3.
M_3	Grow the selected mutants as single plant progenies and check for the segregation of the desired trait.
	Continue selection for the desired trait on a single-plant basis.
	Uniform, non segregating mutant progenies, if any, can be bulked at this stage to speed the breeding cycle.
	Select on an M_2 plant-progeny basis for traits such as resistance to stresses and quality (not reliably expressed on a single-plant basis).
M_4	Evaluate the expression of the selected trait and yield of the bulk lines in comparison with the parent as well as the best check cultivar.
	Record observations on all agronomic parameters, disease and pest resistance compared with the parent variety.
	Repeat the procedure for the single-plant selections, as outlined above,
	Mutants with valuable traits but undesirable characters should be 'backcrossed' with parent or used in crosses.
M_5 generation and beyond	Follow normal plant breeding procedures with the selected progenies: preliminary yield trials, multilocation evaluation of mutants or lines derived from crosses with mutants, submission of one or two of the best lines, at a time, for national or regional evaluation trials, or to local farmers in participatory breeding programmes.
	Initiate seed multiplication to meet the demand of the mandatory trials for official approval and release of the variety.

8.6.6 Oil crop Case study 1: Development of the high yielding oilseed rape variety 'Abasin-95' (Nuclear Institute for Food and Agriculture, Peshawar, Pakistan)

The objectives were to improve productivity and resistance or tolerance to biotic and abiotic stresses.

1988 10 000–15 000 dry seeds of oilseed rape (*Brassica napus*) cv. Tower with 10 percent moisture were irradiated with 1 000, 1 200 and 1 400 Gy gamma rays (60°C), and planted directly in the field in isolation, as M_1. At maturity, four pods from every primary branch were harvested and seeds were bulked on a dose basis.

1989–90 M_2 population was grown in rows and after every 20 rows the parent variety was included for comparison. Individual M_2 plants were selected on the basis of their short stature, early maturity, heavy bearing, long pods, more grains per pod, bold seed, stress tolerance or a combination. The mutant 'RM-152-2' (1 400 Gy treatment) and other mutants were selected, and further advanced to M_5.

1995 RM-152-2 officially released as a new cultivar: Abasin-95.

Two generations a year were grown from M_2 to M_4 to speed up the breeding process (winter in Peshawar; summer in Kaghan).

8.6.7 Oil crop Case study 2: Improving nutrition value.

Another case study describes the re-orientation of breeding objectives for linseed and use of induced mutations as a response to shrinking traditional markets for the traditional oil with good drying properties, as a result of the advent of plastic paints. A programme to change the oil quality from industrial (high linolenic acid content) to edible (low linolenic acid content) was conceived and proved to successful. Various steps—see Table 8.11—applied before and during the programme illustrate well the general principles that should be followed in the use of induced mutations in improvement of industrial crops.

8.6.8 Mutation induction enhanced breeding of asexually or vegetatively propagated crops

According to the FAO/IAEA Mutant Variety Database (MVD, no date), about three-quarters of all released mutant-derived cultivars are sexually propagated species, while only a quarter are asexually or vegetatively propagated (AVP) crops such as ornamentals, trees and shrubs, fruits, root and tuber crops, and sugar cane. Mutation induction shows its most promising aspects in AVP crops compared with cross-breeding methods due to its ability to change only a very few characters of an otherwise good cultivar without significantly altering the remaining, and often unique, genotype. Coupled to biotechnologies such as somatic embryogenesis or micropropagation, mutation induction might be considered an obvious means of conventional plant breeding, and as a possible shortcut for inducing desired genetic alterations in asexually propagated cultivars. Obviously, mutation induction is the only means for producing genetic variability in vegetatively propagated sterile crops and in obligate apomicts (Broertjes and van Harten, 1988).

As good mutation practice, the cultivar to be mutagenized is generally chosen for its outstanding agronomic performance and good adaptation, and the least number of

TABLE 8.11
Breeding linseed (*Linum usitatissimum*) varieties for new uses with altered oil quality

Step	Description of breeding procedure
Screening of crop germplasm	Previous screening for low linolenic acid in linseed germplasm, but none was found.
Screening of wild relatives	Screening in wild species for low linolenic genotypes, and some were found, but crossing with cultivated linseed failed.
Mutagenic treatment of linseed	Seeds of locally adapted variety 'Glenelg' were treated with gamma rays (300 to 900 Gy) and two doses of EMS (0.3 percent and 0.4 percent, 2 h at 2°C and 2 h at 20°C
Screening method development	A rapid, efficient half-seed screening method was developed using a colour reaction.
Mutant screening	Large M_1 and M_2 populations were grown, representative samples of M_2 embryos (seeds produced on M_1 plant) of each M_1 plant were screened.
Mutant confirmation	Reserve half-seeds of those with positive reaction were planted, selected and progeny tested; most proved false positive.
Genetic study	Two independent recessive mutants were induced by 0.4 percent EMS; both showed reduced linolenic acid contents (from 46 reduced to 28 percent linolenic acid) and proved to be at different loci; recombination of the two mutated genes.
Identification of double mutant	Identification of double mutant 'Zero' from recombination of the two single mutants, with only 2 percent linolenic acid (C18:3), and from 19 to 63 percent increased linoleic acid (C18:2).
'Backcrossing' of mutants to parent	Initial mutants showed low yield, and were backcrossed to parent 'Glenelg' to eliminate undesirable mutations. After four BC's, some lines exceeded parent.
Crossing of 'Zero' with other varieties	'Zero' was crossed with a number of varieties and breeding lines, allowing selection of high yielding recombinants with 'Zero' quality.
Release of varieties with altered oil quality	Release of low linolenic acid ('Linola TM') varieties 'Wallaga' (1992), 'Eyre' (1992), 'Argyle' (1994) in Australia; 'Coniston' (1992) and 'Derwent' (1992) in UK; and 'Linola 947' (1993) and 'Linola 989' (1993) in Canada.

Sources: From data presented by A.G. Green (CSIRO, Australia) and co-authors in numerous publications during 1984–2003.

genetic modifications required. Classically, plant material used when treating AVP crops are bud wood, bulbs, corms, dormant cuttings, rooted cuttings, scion wood, stolons, suckers, tubers or even whole growing plants (Table 8.12). AVP crop breeding programmes based on mutation induction face a major technical problem in chimera formation after irradiation of a multicellular meristem. In order to dissociate chimeras easily, the primordia to be used for treatment ought to consist of as few cells as possible. A mutation is a one-cell event, but multicellular apices generally consist of a number of fairly autonomous groups of cell layers, comprising amongst others the epidermis and subepidermis, and have a number of meristematic cells in each layer. More or less small sectors of mutated tissue develop, restricted to one of those cell layers. Thus, chimera formation in most cases results in mericlinal chimeras, which only subsequently develop periclinal branches, shoots or tubers.

Different parameters influence the chances of a mutated cell developing into a sector or layer and to manifest itself. The major one is the position of the mutated cell within the apex. It follows that, after mutagenic treatment and before selection

TABLE 8.12
Radiation doses and plant material used for the induction of somatic mutations in AVP crops

Genus or crop	Plant material treated	Dose
Ornamentals		
Achimenes	Detached leaves	30 Gy
Alstroemeria	Rhizomes	4–6 Gy
Azalea	Rooted cuttings	10–20 Gy
Begonia	Detached leaves	15–25 Gy
Buddleia	Plants	20–30 Gy
Canna	Rhizomes	10–30 Gy
Chrysanthemum	Rooted cuttings	15–20 Gy; 6–12×10^{12}n(th)/cm^2
Clematis	Rooted cuttings	2–5 Gy
Conifers	Rooted cuttings	0.5–5 Gy
Cosmos	Rooted cuttings	20 Gy
Crocus	Dormant bulbs, directly after harvest	10–15 Gy
Dahlia	Freshly harvested tubers	15–25 Gy
Dianthus	Rooted cuttings	40–60 Gy
Dianthus	Unrooted cuttings (base shielded)	80–100 Gy
Endymion	Detached leaves	1–5 Gy
Euphorbia	Rooted cuttings	30–50 Gy
Forsythia	Rooted cuttings	40–80 Gy
Gladioulus	Dormant corms (2n)	40 Gy
Hyacinthus	Bulbs, before-wounding basis	2–5 Gy
Iris	Freshly harvested corms	10 Gy
Kalanchoë	Detached leaves	15–20 Gy
Laburnum	Plants	20–30 Gy
Lilium	Bulb scales	2.5 Gy
Malus	Just-grafted plants	20–30 Gy
Muscari	Detached leaves	10–15 Gy
Narcissus	Dormant bulbs, directly after harvest	5–10 Gy
Ornithogalum	Detached leaves	5–10 Gy
Potentilla	Rooted cuttings	60–80 Gy
Prunus	Just-grafted plants	20–30 Gy
Rhododendron	Rooted cuttings	30–50 Gy
Roses	Budding wood Dormant plants	20–40 Gy 40–100 Gy
Saintpaulia	Detached leaves	30–40 Gy
Scilla	Detached leaves	1–5 Gy
Streptocarpus	Detached leaves	30 Gy
Syringa	Plants	30 Gy
Tulip	Dormant bulbs, directly after harvest	3–5 Gy
Fruit crops		
Apple	Grafts	30–40 Gy 4–7×10^{12} n(th)/cm^2
Banana	Corms	25–50 Gy
Blackberry	Young dormant plants	60–80 Gy
Blackcurrant	Dormant woody cuttings	30 Gy
Cherry	Grafts	20–30 Gy 4–7×10^{12}n(th)/cm^2
Grape	Dormant buds	10–30 Gy
Lemon tree	Cuttings	20–70 Gy
Orange tree	Dormant scions	50 Gy

TABLE 8.12
Continued

Genus or crop	Plant material treated	Dose
Peach	Summer buds	10–40 Gy
Pear	Grafts	40–50 Gy
Plum (European)	Dormant scions	40–60 Gy
Raspberry	Spring suckers	10 Gy
Strawberry	Young runner plant	150–250 Gy
Other crop plants		
Cacao	Buds	10–20 Gy
Cassava	Nodes	30 Gy
Hevea	Dormant green buds	5–20 Gy
Potato	Dormant tuber parts	20–30 Gy
Sugar cane	Buds	20–60 Gy
Sweet potato	Detached leaves	30–40 Gy
Tea	Rooted cuttings	40–60 Gy

can be started, the mutated cells should participate in the formation of a shoot or plant. If some type of adventitious bud technique has been used, many of the mutants will be solid and early selection is possible. Work on irradiated multicellular apices necessitates the mutated sector size to be increased in order to quickly obtain completely homohistont tissue, or at least stable periclinal chimeras. These conditions can be reached in not fewer than 3 to 4 vegetative propagation cycles. The development of advanced *in vitro* techniques, and the extension of these techniques to otherwise neglected species, has created new potentials and opportunities to induce mutations in any AVP crop. *In vitro* plant material, such as apical meristems, adventitious or axillary buds, embryogenic calli, micro-cuttings, cell suspensions or protoplasts, compared with *in vivo* starting material, allows the treatment of larger populations in less space, and plantlets are maintained in a controlled, disease-free environment, facilitating the recovery of mutants. Avoidance or dissociation of chimera and rapid clonal propagation of useful mutants, as well as the production of a large number of plants for further evaluation based on *in vitro* plant material translates into an important gain in efficiency.

Mutagenic treatment

The most common mutagen used with AVP crops is radiation. Bulky material, like bulbs, stolons or scions for grafting, is difficult to treat in a reproducible way with chemicals. The chemical mutagen must penetrate to the meristematic zones, and the excess of chemical has to be removed after treatment. The procedures for acute or chronic irradiation are rather simple, with good repeatability and high mutation frequency. All types of ionizing radiation are effective; in practice, however, it is likely that only an X-ray machine or a gamma-ray source (e.g. ^{60}Co) are available. The dose to be applied depends on the radiosensitivity of the species, cultivar, plant development stage and also of the plant part to be treated. Plant parts that have developed new, adventitious roots and shoots, e.g. unrooted cuttings or freshly detached leaves, are more sensitive than plant parts with existing root and shoot meristems. Thus it is difficult to predict the dose that would be efficient in any new mutation experiment, even for the same cultivar. The best practice is empiri-

TABLE 8.13
Actual *in vitro Musa* mutation induction enhanced breeding process using shoot-tip culture and selection in the field

Steps	Time (months)	Additional information
Sucker from the field Shoot tip culture	T_0	1986 ; cv. Grande Naine (AAA) ; ITC collection
Radiosensitivity test	$T_0 + 6$	1987–1988; FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria
^{60}Co γ irradiation: M_1	$T_0 + 12$	
Micropropagation M_1V_1	$T_0 + 13$	Radiosensitivity tests on a minimum of 200 shoot-tips
Micropropagation M_1V_2	$T_0 + 14$	Irradiation of a minimum of 2000 shoot tips with an LD_{50} dose of γ-rays
Micropropagation M_1V_3	$T_0 + 15$	
Rooting M_1V_4	$T_0 + 16$	
Acclimatization to soil	$T_0 + 17$	1988–1990; line 'GN-60A'; putative early flowering mutant; glasshouse of the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria
Field Selection	$T_0 + 24$	
Stability confirmation and agronomic evaluation	$T_0 + 48$	
Micropropagation of desired plants	$T_0 + 60$	1990–1993; vegetative progeny sent to Honduras, Australia, South Africa and Malaysia for field-testing under commercial plantation conditions. Not all the plants were demonstrating earliness. The chimeric constitution of the original plant could explain this behaviour as only progenies deriving from the mutated sector gave rise to the putative early mutant.
Multilocation trials	$T_0 + 84$	September 1993, in Malaysia, only the early flowering plants obtained in the field were tissue cultured again to produce about 2000 plants for commercial evaluation in the United Plantations Bhd.
Cultivar release	$T_0 + 100$	1995; Malaysia cv. Novaria flowering about 10 weeks earlier than the original parental clone

Notes: LD_{50} = Lethal dose of 50 percent survival; V_x = Vegetative generation.
Source: N. Roux on the basis of his own and F. Novak's experiments at the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria.

cal. Based on empirical data gathered over more than forty years, some dose ranges may be inferred as guidance for a test series (Table 8.12). For vegetatively propagated crops, doses less than LD_{50} are usually employed. A moderate dose that permits good growth and propagation of the material is to be preferred. Too many mutations per cell may be induced at high dose levels, with the risk that a favourable mutation is accompanied by one or more that are unfavourable. In AVP crops, it is very difficult—if not impossible—to separate favourable mutations from unfavourable ones occurring in the same cells because recombination through crossing or selfing is greatly reduced, if not impossible. Which dose level should or can be applied depends on the crop, the type of propagation available, the numbers that can be handled and the selection method.

8.6.9 Case study: Banana mutation induction using in vitro plant material

Based on information from N. Roux on his own and F. Novak's experiments at the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria.

1986 Initiation of shoot-tip culture. Suckers from the field of cultivar 'Grande Naine' (AAA), ITC collection.

1987–88 Radiosensitivity tests on a minimum of 200 shoot-tips, ^{60}Co γ irradiation, M_1, micropropagation M_1V_1 to M_1V_3, rooting M_1V_4 plants.

1988–90 Acclimatization to soil, field evaluation, selection of 'GN-60A,' putative early flowering mutant, stability confirmation and agronomic evaluation in glasshouse of the FAO/IAEA Laboratory.

1990–93 Micropropagation of selected plants, multilocation trials, vegetative progeny sent to Honduras, Australia, South Africa and Malaysia for field-testing under commercial plantation conditions. Not all the plants of GN-60A were demonstrating earliness. The chimeric constitution of the original plant could explain this behaviour, as only progenies deriving from the mutated sector gave rise to the putative early mutant.

1993 In Malaysia, only the early flowering plants obtained in the field were tissue cultured again to produce about 2 000 plants for commercial evaluation in the United Plantations Bhd.

1995 Cultivar 'Novaria' released in Malaysia, flowering about 10 weeks earlier than the original parental clone.

$V_{\#}$ = Vegetative generation

Because the integrated use of mutation induction and *in vitro* technology speeds up the whole procedure, it is possible to increase the propagation rate and generations per unit time and space, and thereby enhance the economic efficiency of the process. However, some bottlenecks remain. With the increased use of shoot-tip culture for *Musa* micropropagation, somaclones are being detected among regenerated plants. This—mostly undesirable—variation interferes with the induced mutations and makes the selection of useful mutants more difficult. The actual process is summarized in Table 8.13.

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CHAPTER 9

Selection methods Part 1: Organizational aspects of a plant breeding programme

Salvatore Ceccarelli

9.1 INTRODUCTION

The topics covered in this chapter are seldom described in standard plant breeding books, as they arise after the basic choices have been made concerning the breeding programme, such as the choice of germplasm, the choice of the breeding method(s), and the choice of experimental designs and of statistical analysis. We will first analyse the organizational aspects of centralized breeding programmes (CBP), defined as breeding programmes entirely conducted in one or more research stations except for the testing of the final products. We will then examine the organizational aspects in the case of decentralized breeding programmes (DBP): these are defined as breeding programmes in which selection and testing are conducted outside the research station and in the target environment. Subsequently we will discuss the organizational aspects of decentralized-participatory breeding programmes (DPBP), which are defined as breeding programmes in which selection and testing are conducted in the target environment(s) with the participation of the users. One important aspect in the organization of a breeding programme, namely priority setting, has already been discussed in Chapter 4.

9.2 CENTRALIZED BREEDING PROGRAMMES

In the case of a breeding programme conducted entirely on one or more research stations, the organizational aspects are affected by a number of variables, which are both predictable (they tend to come up every year, a typical example being budget changes) and can be addressed within the framework of the research station management, as well as unpredictable variables, such as staff resigning from the job. Several of these variables are common regardless of the type (CBP, DBP or DPBP) of breeding programme.

The organizational aspects that are discussed are:

- land allocation and use (choice of rotations, input levels, depth and time of planting, etc.);
- organization and management of physical resources (equipment);
- organization of human resources (technical staff and labour);
- data capture, storage and analysis; and
- farmer participation in a CBP (farmers visiting and selecting from on-station trials).

9.2.1 Land allocation

One of the major organizational issues in managing a plant breeding programme within a research station is the allocation of land, because, usually, more that one breeding programme operates within the same station, alongside other research programmes, and therefore competition for land is common. The amount of land available to a breeding programme affects a suite of choices ranging from the experimental design, number of replications, plot size and last but not least, the type of rotation under which the material is tested. As nearly all research stations do some type of commercial crop production, the rotation, under which the breeding material is tested, is not chosen based on scientific consideration but on which crops are expected to generate the highest income.

Furthermore, if the only choice left to the breeder is to follow a crop grown for commercial purposes, this also implies testing the breeding material under levels of fertilizers and other agricultural inputs (pesticides, herbicides, etc.) that could be difficult to justify when breeding is for typically low-input crops. Therefore, these

organizational issues limit the freedom of the breeder in terms of breeding strategies (for example, the choice of testing the breeding material under a given level of inputs). The only advantage of following a commercial crop on station is that a commercial crop is expected to leave the land highly uniform. However, this is not necessarily true because, for example, the uneven application of inputs can actually create additional sources of uncontrollable variation.

A much better type of organization is when each breeder is allocated two to three times the amount of land needed for the breeding trials and nurseries in a given cropping season to be able to manage, according to their breeding philosophies and strategies, not only the portion of land allocated to current trials and nurseries, but to manage (in terms of rotations and inputs) also the land that will host trials and nurseries in one or two years. This improves considerably the situation compared with the organization described earlier, but it is not without negative aspects. The breeder has often to produce and store, or to purchase, the seed for the cover crops to precede the trials, and has to supervise the agronomic operations to make sure that the complex of rotation and management under which the cover crop is grown represents exactly the conditions under which they intend to test the breeding material.

Even when breeders have full control of the land and of the agronomic operations, the situation on the research station will never be able to represent the variable agronomic situations under which the crop is actually grown by farmers. Often, particularly in developing countries, farmers grow the same crop in a number of different rotations and with different levels of inputs depending on the environment and on the wealth of the farmers and their access to the market. For these reasons, some breeding programmes do extensive evaluation, selection and testing in farmers' fields. Even though the choice of evaluating and testing the breeding material in farmers' fields has nothing to do with participatory plant breeding (PPB) (it could possibly be considered participatory variety selection – PVS), it is expected to have a number of advantages over on-station evaluation, selection and testing. These advantages derive from exposing the breeding material to a multitude of target production areas at an early stage of the breeding programme, assessing the response of different breeding material to a range of different soil types, soil depths, rainfall, agronomic management, etc.

The theoretical framework for discussing response to decentralized selection and more generally the optimum environment for selection, was developed by Falconer (1952, 1981), who demonstrated that selection in the target environment is almost invariably more efficient than indirect selection, i.e. selection in a different environment. This has been confirmed by the theoretical work of Rosielle and Hamblin (1981) and Simmonds (1991), and validated by data from numerous experiments, reviewed by Ceccarelli (1996).

At the same time, the superior efficiency of selecting in target environments has also been disputed by several scientists. However, in the majority of cases (such as Atlin, McRae and Lu, 2000; Rizza *et al.,* 2004; Dodig *et al.,* 2008) this was based on data from a narrow range of yield levels (see also Chapters 2 and 20).

It is important to specify that all breeding programmes have some degree of decentralization in the sense that, sooner or later, the breeding material is tested outside the research station. However, we restrict the use of the term 'decentralized breeding'

to mean decentralized selection (Simmonds, 1984), as opposed to decentralized testing, which is commonly the last stage of any breeding programme.

Before we examine the organizational issues associated with a DBP, we need to clarify that a breeding programme is not always taking full advantage of operating in farmers' fields. Cases where the trials are planted in farmers' fields under rotations used in the past but discontinued, or with an unrealistically high level of inputs, or placed at the bottom of a slope where water harvesting effects create a unique micro-environment, indicate that decentralization does not always and invariably mean a higher relevance of the results for the final users.

As we will see later, the management of physical resources is a major issue in participatory breeding programmes dealing with crops or countries with full mechanization, while is much less of an issue with crops or countries where hand operations prevail.

9.2.2 Organization and management of physical resources (equipment)

Similarly to land allocation, physical resources such as vehicles, plot equipment (drills and combines), seed cleaners and seed dressing equipment are in some cases kept in a pool and in other cases assigned specifically to a given breeding programme. The first options is usually favoured by administrators for the most efficient use of physical resources, while the second is favoured by breeders because it avoids additional bureaucratic layers and forms to fill, and it makes sure that the equipment is always available when it is needed. The second option has the advantage of generating a sense of ownership (lacking in the first option), with beneficial effects in terms of care and maintenance.

When the administrators are able to create a healthy working environment with good cooperation between breeding programmes, resulting in sharing and exchanging equipment, the second option can be nearly as efficient as the first one.

9.2.3 Organization of human resources (technical staff and labour)

The management of the human resources (research support staff and labour) associated with a breeding programme is one of the most challenging organizational issues, because the quantity and quality of the work depends largely on their performance. Potential sources of errors are very many in a breeding programme, starting from arranging seed envelopes according to the randomization plan, filling them with seed, planting according to the experimental layout, note taking, harvesting, storing the seed while data are analysed, retrieving the seed of selected entries, and storing the seed till the following planting season. One of key questions in organizing the research support staff around these several tasks is whether to have each staff member assigned to one or more specific task, or to have all of the staff able to perform every operation in the breeding programme.

The first option is usually preferred by the support staff because it is associated with the professional end-of-year evaluation. The major risk associated with this option is the gap of expertise which is created in the case of staff being absent for a long period of time, or even leaving permanently.

The second option has the advantage of greater flexibility in organizing the work and of creating a wider spectrum of prospects should staff leave the breeding programme and apply for other jobs. One exception could be the responsibility for data handling and data management, which is usually the responsibility of a single

support staff member, but shared with the breeder(s) (see also the section below).

9.2.4 Data capture, storage and analysis

The traditional manner of organizing data capturing is the manual recording through field books. Field books can be produced using specialized software tools, such as AGROBASE™ (www.agronomix.mb.ca), Excel™ or databases such as Access™. Manual capturing of data has a number of disadvantages, including:

- the preparation of field books is time consuming;
- note taking is weather dependent (field books are very difficult to use on windy or wet days);
- the data are handled twice, being written in the field book first and entered in the computer later, thus increasing the probability of manual errors; and
- the time required for data entry delays statistical analysis, usually until after harvesting, hence reducing the possibility of detecting errors by examining the results of an analysis conducted immediately after the data are collected.

Today data capturing can be easily done electronically using palmtops (there are very many types available on the market) or specifically designed devices, which are usually much more expensive. The file, which will normally be printed as a field book when data capture is by hand, is downloaded into the main memory or in the flash card (recommended) of a palmtop (they usually handle a variety of file types, depending on the brand), which can then be taken to the field to enter data. Electronic data capture has a number of advantages:

- data are entered manually only once and then transferred electronically to the main computer for analysis;
- before leaving the field, it is possible to quickly check the data through sorting and ranking, and immediately correct typing mistakes;
- data can be collected in the field under a wider range of climatic conditions than with field books;
- data analysis can immediately follow data collection, thus providing an additional means of checking for errors in data entry while the crop is still in the field; and
- use of memory cards enables one to keep at hand in the field all the relevant information concerning all trials and nurseries in a large breeding programme.

At the end of the season it is always possible to produce a printout of all the files and to maintain a hard copy of all the data.

Safe data storage is a major issue in plant breeding programmes. Examples of strategies that can be used to reduce to a minimum the risk of data loss are frequent backup; storage of data in external disk drives; and storage of data in at least one computer never connected with networks or the Internet to reduce the probability of introducing viruses.

9.2.5 Farmer participation

Farmer participation in a CBP (farmers visiting and selecting from trials on-station) is not included as a section under PPB because farmers being invited to a station to select between lines, hybrids or clones do not have a chance to develop any sense of ownership of the material they select, which is usually associated with participation in a cyclic, as opposed to linear, process (see also later). Therefore, as noted earlier, farmer participation in a CBP is more akin to PVS than to participatory plant breeding.

As practical experience confirms, farmer selection is environment-dependent (Ceccarelli *et al.*, 2000), so farmers par-

ticipating in on-station selection should be invited from areas that are similar to the research station in terms of the climatic, agronomic and agricultural systems when the breeding programme has only one station. When the breeding programme has a number of stations simulating a number of different environments, different groups of farmers should be invited to different research stations, unless this activity is part of a research activity involving all farmers making selections on all stations for the sake of comparison.

As farmer visits are usually a single event, the organizational issues involve transportation of the farmers to the station and back, and possibly accommodation for those farmers coming from far away. The logistics can be simplified by inviting only nearby farmers.

Farmers' visits to research stations are a typical activity of the research programmes (not only breeding) in developed countries, where logistic problems are much reduced as farmers are usually able to travel to and from the station by themselves.

If the visit aims at farmers selecting finished or nearly finished varieties, other organizational issues include whether the farmers give a formal score to the breeding material; whether and how the breeders use farmers' preferences as an additional selection criterion; whether and how the material selected is made available to the farmers; and what follow up activity will occur.

9.3 DECENTRALIZED BREEDING PROGRAMMES

Transferring a breeding programme to outside a research station almost always implies losing some degree of control of a number of steps and operations. This is often associated with the perception that less control by scientists is associated with less precision, and this explains the reluctance with which several plant breeders, particularly those in the developing countries, operate away from their research stations.

Within a research station, all the operations associated with running a breeding programme are shared by staff belonging to the same institution and having daily interaction (which does not necessarily make things easier). When a number of stages are transferred outside the research station, a number of operations can, and actually should, be shared with staff belonging to other institutions or to out-posted staff of the same institution, or a combination.

Depending on the presence or absence of a strong extension service, and of the structure of the research institute responsible for the plant breeding, a number of different scenarios are possible.

In the case of countries with a strong extension service and the presence of regional (or subregional or provincial) research centres with infrastructure such as offices, computer facilities, agricultural equipment (including plot machinery), a DBP could be organized based on the following principles:

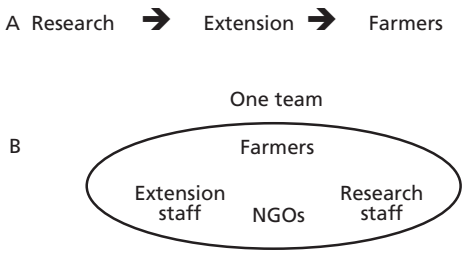
- The scientist(s) at the institute's headquarters are responsible for the preparations of trials (choice of entries, plot size, experimental design, and having the seed in envelopes ready for planting), the preparation of field books (or electronic files for electronic capture of field data), the preparation of draft field maps with possible alternatives for the layout of the trials, and the shipment of trials with all the detailed instructions for planting and note taking.
- At the headquarters there will be a central database where all the information generated in the breeding programme is kept. Information generated in the regional

centres should also be kept where it was generated as a form of safety duplication.
- The main responsibility of the staff of the extension service is to collaborate in the selection of the sites and the specific fields, according to the type and objectives of trials and the general philosophy of the breeding programme.
- The research staff in the regional centres is responsible for implementing the trials on the ground, ensuring the required management, the timing of the field operations and eventually for collecting field data, which are then transferred to headquarters for statistical analysis. Alternatively, when the necessary expertise is available, they can be requested to do the single site analysis, leaving the responsibility for the multi-site analysis to headquarters.
- Extension and research staffs are also responsible for the organization of field days. These are useful not only to show the potential clients the new breeding material, but also to particularly understand through the interaction with farmers whether the experimental setting (location, type of soil, type of management, etc.) is actually representative of farmers' conditions.

This overall organization is facilitated by involving all staff participating in the implementation of the breeding programme in regular meetings, through which the basic principles of the breeding programme are understood and shared by everyone. This obviously includes the full sharing of results among all the participants on an annual basis.

One important beneficial effect of this type of organization is that it replaces the traditional linear sequence of information typical of agricultural research with a continuous flow of information between the different partners (Figure 9.1). As we will see later in this chapter, this concept is fully developed in a participatory breeding programme.

FIGURE 9.1
Replacing the linear sequence Research > Extension > Farmers (A) with continuous flow of information between the different partners (B)



The figure hypothesizes a general situation with a multitude of partners, some of whom may not be present in specific situations.

In this type of scenario, one of the main sources of additional cost associated with decentralized breeding, i.e. transportation and travel, is considerably reduced.

In the case of countries where the extension service is limited or absent, all the responsibilities have obviously to be borne by the research staff.

In describing the organizational aspects of a DBP we are deliberately ignoring the use of additional research stations as 'decentralized' sites, because, even if substations capture differences in temperature and rainfall, they still suffer from all the management issues described earlier, and therefore they may not represent any real production environment.

9.3.1 Countries with only the selection and testing stages of a breeding programme

A special case is that of those countries where, for various, reasons, the national breeding programme cannot afford to go through

the first stage of a breeding programme (Chapter 3), i.e. the generation of genetic variability (regardless of the method), and therefore relies entirely on either locally collected germplasm, or on germplasm donated by breeding programmes in other countries or other research centres, such as international agricultural research centres, or by a combination of the two. In such cases, the research station should be used for seed multiplication and also for negative selection, particularly in the case of introduced germplasm, which might have photoperiod or vernalization requirements that makes it ill adapted for national conditions.

Seed multiplication is necessary because the seed from germplasm collections is usually in very small quantities, as it is generally the amount of seed of some of the breeding material received from other breeding programmes.

The steps following the initial seed multiplication depend on the breeding methods and on the type of genetic material received or collected, but will vary from a centralized, on-station, selection evaluation and testing, with only the final stages transferred to farmers fields, to a decentralized programme of the type described above, or to a fully DPBP.

9.4 DECENTRALIZED-PARTICIPATORY BREEDING PROGRAMMES

At the beginning of the chapter we defined participatory plant breeding programmes as breeding programmes in which selection and testing are conducted in the target environment(s) with the participation of the users. Here we will add that, in order to reach its maximum effectiveness, the participation of users should take place as early as possible, and ideally at the beginning of stage two in a plant breeding programme, as described in Chapter 3.

The organizational aspects of a decentralized participatory plant breeding programme do not differ conceptually from those of CBPs. The major difference is that the decisions and the choices for the organizational aspects involve all the stakeholders, and the type of participation depends on how, when and which stakeholders are involved.

We will examine the following organizational aspects:

- Setting criteria to identify target environments and target users.
- Users (different uses of the crop, gender, age, wealth, etc.).
- Locations (representativeness, relevance for the crop, different agroclimatic environments).
- Identification of the target environment and users.
- Type of participation.
- Choice of breeding method.
- Management of trials in farmers' fields.
- Type of genetic material, field layout, machine vs hand operations, data analysis, multi-environment trials (MET).
- Institutionalization of participatory plant breeding.
- The transition phase.

We will not cover here the organizational aspects of Variety release and seed production (Countries with and without a formal seed production system) as these are covered in Chapter 21.

As mentioned earlier, some of these organizational aspects are common to all breeding programmes, while some are specific to a DPBP. At the end of the chapter we will discuss the organizational changes required to migrate from, for example, a centralized non-participatory breeding programme to a DPBP

9.4.1 Setting criteria to identify target environments and target users

A decentralized participatory plant breeding programme may lose a great deal of its potential effectiveness if the sample of both environments and users in which the programme is implemented do not represent both the target environments and the target users. In order to do that, setting the criteria for identification of the target environments and users is a critically important step.

In setting the criteria, it is useful also to assign priorities to the different categories of environments and users so that, depending on the resources available to the programme, environments and users can be added or discontinued on the basis of priorities established in an ideal context.

The most obvious criterion for the choice of the target physical environments, is their representativeness of the major production areas for a given crop (or for the crops covered by the programme) in terms of climatic conditions (temperature, rainfall, elevation), agronomic practices, soil types, landscape, etc. The criteria for the choice of the socio-economic environments are closely interconnected with those of the target users. Therefore the programme has to decide whether to work for all the various socio-economic environments present in the target area, or to privilege the most difficult environments where farmers have fewer opportunities for market access and where most of the agricultural products are used within farms or within the community, or to work only for the most favourable, high potential, environments. It has been argued that PPB has evolved mainly to address the difficulties of poor farmers in developing countries (Ashby and Lilja, 2004) which have been largely bypassed by the products of conventional breeding. In fact there is no reason why the approach should be confined to work with low-income farmers. Basically, when done properly, PPB is an approach that, even if applied in a variety of modes, merges the technical knowledge of the 'scientists' with the knowledge of the 'farmers', which is historically based on millennia spent in domesticating wild plants and adapting the resulting crops to a multitude of different environments. Therefore, in principle, PPB can apply equally well even in situations of market-oriented agriculture in favourable environments.

The main criteria to identify farmers can be grouped in three broad categories:

- ***Farmer characteristics*** These include language, ethnicity, caste, age, gender, income, education, market relations or orientation, membership of farmer organizations (unions or cooperatives), and relationships among groups within the same community and between communities.
- ***Farmer expertise*** This includes the need to understand whether farmers are already practising some types of plant improvement, as this is essential in the choice of the breeding methodology (see below). In some communities, e.g. Eritrea, specific individuals have specific responsibilities in relation to crop and variety introduction (Soleri *et al.*, 2002).
- ***Farmer needs*** These include the needs of different groups, their perception of risk and hence the type of variety they consider more appropriate in term of stability and yield (Soleri *et al.*, 2002), and the need for special quality attributes either for feed or for food, or both. These include also the farmers' understanding of production limitations with reference to the use of fertilizers, appropriate rotations and irrigation. It is also important to understand farmers' needs in terms

of seed supply, because it makes a large difference whether the farmer predominantly use their own seed (or the seed of their neighbours), or usually buy seed from the formal sector.

9.4.2 Identification of the target environment and users

Once the criteria are set, the actual process of identification needs the involvement of partners who have a very good knowledge of both the environment and the users. These are typically the staff of the extension service or the staff of the outlying research stations. The first step is to set meetings with all the stakeholders with the objective of identifying partners and locations.

In this phase there are some potential biases that can affect the success of PPB. Key decisions affecting the participatory programme are (i) whether to seek individual or group participation; (ii) whether the participants should be experts (germplasm experts are farmers who regularly experiment with varieties, are able to recognize important intra- as well as inter-varietal differences, and who target specific varieties to different micro-niches) or whether they should represent the wider community; and (iii) whether equity should be the main objective in the identification of the users. Meetings with all different typologies of farmers may be inappropriate without a proper knowledge of the power relationships within the community. This usually leads to a few farmers monopolizing all the discussions reducing the possibilities for others to express their views. This danger varies greatly with the culture: in some cultures, women are not even allowed to attend meetings; in others, they can participate with a passive role; and in others they can participate freely and with the same rights as the men. Therefore, it is not possible to give a 'cookbook formula' for what works better. In general, if some groups or individuals tend to be discriminated against, it may be appropriate to have separate meetings with different social, gender, age or wealth groups.

In the process of identifications of users, it is very important to clarify (i) what plant breeding can offer and how long it can take; (ii) what sort of commitment in land, time and labour is required from the farmer; (iii) what is the risk for the farmer and how this can be compensated for (in-kind compensation vs. money), and (iv) what the overall benefits are that the farmers can expect if everything goes well.

In these meetings it is also essential to understand what sources of seed farmers use for the various crops, to anticipate which type of change the participatory programme might introduce, and to make sure that farmers are aware and prepared to absorb the changes.

The organizational issues of the choice of sites are both at the macro- (identification of villages or locations within a country or a region) and at the micro-level (identification of the field within a village for planting the trial(s). The participation of farmers in the identification of the fields is unavoidable because it is associated with the relevance of the results and with the issues of 'who participates' and 'who benefits': it is at this point that small-scale farmers run the risk of being excluded as active participants because their land is not large enough to host trials in addition to the farmers' crop. As we will see later, it is possible to find experimental designs that allow distributing relatively large number of entries in small blocks, each planted in a different farmers' field.

An additional organizational issue in the choice of the sites, which is associated with

the issue of the breeding philosophy, is whether they should be sufficiently representative to allow some degree of extrapolation of the results to other sites, or whether the priority should be to meet farmers' needs to target micro-niches. In practice, it is advisable that sites do represent the range of environmental and agronomic conditions in which the crop is grown, because this is known to have a major effect on farmers' selection (Ceccarelli *et al*., 2000, 2003).

Participatory breeding programmes are often seen exclusively as programmes leading to niche varieties, adapted to only a restricted complex of environmental and social characteristics (see also Chapter 4). This is not necessarily true, as the type of adaptation (narrow or wide) of the varieties emerging from a PPB programme is largely dependent upon the nature of the locations and the users. If the locations covered by the programme represent a mix of favourable and unfavourable growing conditions, it may be expected that the more uniform environmental conditions that generally characterize favourable environments will led to the selection of the same varieties across a number of locations (widely adapted in a geographical sense), assuming that farmers' preferences are also homogenous across the same locations. In the more unfavourable conditions, one can expect that more location-specific varieties (narrowly adapted) will be selected. Eventually, even if the selection is conducted independently in each of many locations, giving the impression that selection is for specific adaptation, the process will not discard a truly widely adapted genotype if such a genotype does exist in the breeding material (Ceccarelli, 1989). Therefore a PPB programme easily results in a mixture of widely and narrowly adapted varieties.

What is discussed above also depends on the definition of wide and narrow adaptation. Narrow and wide are relative terms; therefore, for an international breeding programme, a widely adapted variety is a variety performing well in a number of countries, while for a national breeding programme it is a variety performing well in several locations within the country, while, ultimately, to a farmers it means a variety performing well across cropping seasons – without too much concern whether it performs well elsewhere.

It is difficult to reach an optimal allocation of resources regarding to the number of sites and to the number of farmers at each site. As we will see later, it is possible to organize a PPB programme in such a way that G×E interaction, and more specifically Genotype × Location (G×L) and Genotype × Years within Locations (G×Y(L)) will eventually optimize the overall structure, at least from a biological point of view. This aspect is covered in depth in Chapter 20.

9.4.3 Type of participation

Several scientists (Biggs and Farrington, 1991; Pretty, 1994; Lilja and Ashby, 1999a, b; Ashby and Lilja, 2004) discriminate among different types or modes of participation, which are not necessarily mutually exclusive, although there may be trade-offs among the impacts of the different types. Based on two groups of decision-makers, namely 'scientists', which includes research programmes and extension agencies, and 'farmers', which refers to the intended users of the participatory breeding programme varieties, PPB is categorized by Ashby and Lilja (2004) as:

- ***Consultative*** Scientists make the decisions alone, but with organized communication with farmers. Decisions are not made with farmers nor delegated to them.
- ***Collaborative*** Decision-making authority is shared between farmers and scientists

based on organized communication between the two groups. Scientists and farmers know about each other's ideas, hypotheses and priorities for the research through organized two-way communication. Plant breeding decisions are made jointly; neither scientists nor farmers make them on their own. Neither party has the right to revoke or override the joint decision.

- ***Collegial*** Farmers make plant breeding decisions collectively, either in a group process or through individual farmers with organized communication with scientists. Farmers know about scientists' priorities and research hypotheses through organized one-way communication. Farmers may or may not let this information influence their plant breeding decisions.

Ashby and Lilja (2004) also recognized *Conventional* (no farmer participation) and *Farmer experimentation* (no scientist participation; most of the pre-1900 breeding was of this type) as two additional typologies of PPB. In the first, scientists make the decisions alone without organized communication with farmers, while in the second, farmers make all the decisions, either as a group or as individuals, on how to experiment, introducing new genetic material without organized communication with scientists.

We will not discuss these last two typologies any further, because they represent two types of plant breeding that explicitly exclude participation of either one or the other of the two essential partners.

Two other two categories of PPB were defined by the Plant Breeding Working Group (PBWG) of the System-Wide Programme for Participatory Research and Gender Analysis (SWPPRGA), and by McGuire, Manicad and Sperling (1999), Weltzien *et al.,* (2000, 2003) as *Formal-Led PPB* when farmers join in breeding programmes which have been initiated by formal breeding programmes, and as *Farmer-Led PPB* when scientists seek to support farmer's own systems of breeding, variety selection and seed maintenance.

In practice, field experience indicates that PPB is a continuously evolving process. It is quite common that, as farmers become progressively more empowered—an almost inevitable consequence of a truly participatory breeding programme—a consultative programme gradually evolves into collaborative and collegial. Similarly, a Formal-Led PPB can gradually evolve into Farmer-Led PPB, and could eventually be entirely handed over to farmers.

9.4.4 Choice of parental material

The choice of parental material is of critical importance in a breeding programme and in this book is covered in Chapters 3 and 6. Here we only add that, as in a conventional breeding programme, the parental material in a participatory breeding programme is, with few exceptions, the best material selected, by farmers in the case of PPB, in the previous cycle.

9.4.5 Choice of breeding method

The breeding method is only one of the factors determining the success of a breeding programme; the others include the identification of objectives and the choice of suitable germplasm (Schnell, 1982).

In conventional breeding programmes, the choice of the breeding method is purely the responsibility of the breeder and is largely affected by the breeder's scientific background and by the mandate of the organization, public or private, for which the breeder works.

In PPB, the choice of the breeding methods can not be made without

considering whether and how farmers are handling genetic diversity. The rationale is as follows. As described in Chapter 3, the generation of variability is the first step of any breeding programme, conventional or participatory, followed by the utilization of variability and eventually the testing of the prospective varieties. In a number of countries, farmers do use genetic diversity either as a specialized activity within the community, or as an individual initiative (Chapter 22).

For example, in Eritrea it is common for farmers to select individual heads within a wheat or a barley plot, plant them as head rows in a small portion of their field, decide whether to bulk one or more rows and start testing the bulk in the field of other farmers, initially on a small scale and gradually on a larger area. One of the most widely grown wheat varieties in the country has been developed starting from a small seed sample bought by an expert farmer in a local seed market and planted initially as spaced plants. In Nepal, before harvesting the crop, farmers growing the old barley landraces habitually collected a sample of heads representing all the different morphological types present in the field to produce the seed to be planted in the following cropping season. In contrast, in the Syrian Arab Republic and in many other countries in the Near East and North Africa, the selection unit is a plot, and excessive heterogeneity within a plot not only is not exploited, but is also considered undesirable.

These three examples indicate that, even within the same crop, a participatory breeding programme has to use different breeding methods, at least at the beginning of the programme, to ensure full participation. It is obvious that a blanket approach, based on the same breeding method used everywhere regardless of whether and which skills farmers have in handling genetic variation, can not ensure true participation, as farmers will be confronted with methodologies they can not relate to anything with which they are familiar.

In addition to the examples given earlier, breeding methods may differ for the same crop within the same country. Using Africa as an example, barley is grown in Ethiopia and Eritrea both as food and feed (largely landraces) and also for malt production for local breweries. While population methods can well be used in the first case, pedigree breeding is suitable in the second.

An issue related with the choice of the breeding method is how much breeding material farmers can handle. This is a controversial issue, and several scientists believe that farmers can only handle a very limited number of genotypes and therefore, implicitly, believe that the only form of participation is PVS. If true, this will make it impossible to implement true PPB programmes, because plant breeding needs to start from a sufficiently large sample of genetically variable material.

Field experience shows that when discussing the number of genotypes farmers can handle, it is very dangerous to make assumptions before discussing the issue with the farmers.

The choice of the breeding method also depends of the genetic structure of the final product, i.e. pure lines, mixtures, hybrids or open pollinated varieties. It is important to note that farmers can change the type of final product originally planned by the breeder. For example, in Syria, where, in the case of self pollinated crops, the formal system only accepts pure lines for release, farmers do not mind adopting bulks as long as they are not too heterogeneous. In the case of barley, we also have the example

of one farmer testing the advantage of a mixture of a 6-row genotype, adapted to high rainfall and lodging resistant, with a 2-row genotype adapted to low rainfall and lodging susceptible. Similarly in Egypt, we found that farmers plant a mixture of all the lines selected one year earlier (Grando, pers. comm.).

In principle, all breeding methods can be employed in PPB, keeping in mind that 'participatory' does not mean that ALL the breeding material has to be planted in farmers' fields. Several examples of different breeding methods used in actual participatory breeding programmes can be found in Almekinders and Hardon (2006).

Given that plant breeding is a cyclic process (see Figure 9.2), one organizational issue that is often debated is the stage of the plant breeding programme at which participation should start. This issue in effect makes the difference between PPB and participatory variety selection (PVS; see Chapter 3, section 3.7), where the participation of farmers takes place during the third stage of the breeding process, after the genetic variability available at the beginning of the cycle has been—usually—drastically reduced. We believe that farmer participation should, at a minimum, coincide with the second stage of a breeding programme, possibly when the genetic variability is still at or near its maximum. There are examples of PPB programmes where farmers can start as early as making crosses, such as the PPB rice programmes in Bhutan and Viet Nam (SEARICE, 2003), which does not necessarily imply emasculation and manual pollination, but, for example, mixing different genotypes or cultivars of cross-pollinated crops to facilitate intercrossing. Even when they do not manually make the crosses, in a PPB programme that runs over cycles of selection and recombination like any other plant breeding programme, farmers control the crossing programme by selecting the best entries, which are usually the parents of the following cycle, as discussed earlier under choice of parental material.

Eventually, a breeder planning to start a PPB programme is faced with the issue of whether the breeding method used in a non-participatory programme needs to be changed. While there are breeding methods that are easier to fit into a participatory context, a breeder does not have necessarily to change the breeding method, given what was said earlier about fitting the method to whatever type of breeding farmers are already doing. Here, we might add that, like other aspects of PPB, the methodology can also evolve as new farmer skills emerge. Several examples can be found in Almekinders and Hardon (2006).

9.4.6 Management of trials in farmers' fields

The organizational issues of implementing trials in farmers' fields differ considerably from those in a research station, and are more similar to those of a DBP. However, they diverge significantly from a DBP when farmers take full responsibility for planting and harvesting.

The first differences are issues such the choice of the actual portion of land on which to plant the trial, the total number of plots in each trial, the type of controls (check varieties), the plot size, the seed rate, the distance between rows, the dates of planting and harvesting: all these have to be discussed with each community in each location. It is not simply a matter of courtesy. Farmers' interest in the trial is directly proportional to their participation in its design and management. The inability of the scientists to accommodate farmers' requirements may lead to a total lack

of interest by the farmers. For example, in the case of barley in the Syrian Arab Republic, farmers believe that seed rate is extremely important. Whether this belief is correct or not is immaterial, because if the scientists use the seed rate they believe right, farmers may even refuse to carry on selection. Therefore, in the participatory barley breeding programme in the Syrian Arab Republic, for example, we are using as many as eight different seed rates, ranging from 50 kg/ha to 250 kg/ha. As this is believed by the farmers to have a major effect on barley yields, an important side-activity would be the visits of farmers to locations where a very different seed rate is used; this might be the best way to generate an interest in testing alternative seed rates.

One fundamental principle in discussing organizational issues with farmers' communities is to pose and justify the problem, not to present a solution. The solution should come from the community, and if the community or the individual farmers are not prepared to solve the problem, a possible solution can be offered, but only as a suggestion.

The choice of land, which in a CBP usually depends on the farm manager, in the case of a DBP (whether participatory or not) has to be agreed on by the farmer. It has to represent a suitable rotation and a good uniformity (this should be checked the year before, together with the past history of the field). The size required by the trial may not match that allocated by the farmer to that specific rotation. In this case, the extra land has to be planted by the scientists with a cover crop using a variety chosen by the farmer.

The type of genetic material to be used in the programme needs to be discussed with farmers. Initially, the scientists may find that farmers are not aware of the diversity within the crop, and in this case our suggestion is to start with a wide array of genotypes representing as wide range of diversity as possible. But there are cases where farmers have previous experience with various type of germplasm and they may feel very strongly concerning one or more types of specific germplasm type. For example, in he Syrian Arab Republic, farmers grow two landraces: one with black seed, which is grown predominantly in dry areas, and one with white seed, which is grown predominantly in wetter areas. Farmers feel very strongly about the seed colour and therefore in the participatory barley breeding programme in the Syrian Arab Republic we make available different initial genetic material in the two areas. The issue of the type of genetic material covers also the issue of the checks. The checks have the dual purpose of providing an estimate of error variance (for example, in unreplicated trials with systematic checks) and to provide a comparison for farmers during selection. The ideal solution is to have a well adapted variety to fit both purposes, and if the choice of the check(s) is left, as it should be, to the farmers, this is usually their choice.

The issue of managing the equipment in a PPB programme is similar to a DBP. If the country has a network of research stations each with its own equipment, it is obviously more economical that each station uses its own equipment for all the field operations. Where machinery has to be moved from one central research station to all the trials sites, the number of sites and of trials has to be adjusted to allow all the necessary operations to be performed in time. Usually farmers are extremely concerned about planting and harvesting at the right time, and if the choice is between having several locations and being late in

both planting and harvesting in some of them, it is advisable to reduce them to a number that can be managed properly. The issue of timely harvesting, in the case of completely mechanized crops, can be solved by estimating yield through a hand-harvested sample of the plots. This has the additional advantage of estimating the total biological yield, a character of major importance in many developing countries. The farmers can then harvest by combine whatever is left in the field. This of course assumes that the seed requirements for the following year are satisfied. The need for timely planting and harvesting makes it much easier to organize a PPB programme in countries or for crops where both planting and harvesting is done by hand. In this case, the scientists can limit themselves to the preparation of the trials, visit each site to show the trial layout, leave the envelopes or the bags properly numbered, and let the farmers do the planting themselves, as it happens in a PPB programme for barley in Iran.

The issue of managing the equipment in a situation of fully mechanized operations can also be addresses by empowering farmers to conduct trials. This often poses technical challenges, because commercial drills and combines are not suitable for planting experimental plots.

Finally, two additional issues in managing trials in farmers fields concern the physical layout of the trials, and the management of crop residues, border rows and leftovers (in the case of sampling).

In arranging the trials on the ground, two principles are important: the first is that no land should be left uncultivated. In many farming communities in developing countries, leaving even a few square metres of land uncultivated is considered almost a crime, and this is particularly true in marginal and dry areas where yield per unit of land is low. Therefore, no gaps should be left between plots, as is common practice on research stations to facilitate the identification of plots, and the alleys should also be planted. To facilitate farmers during selection, and to avoid seed mixture if the seed from the trial is to be used the following year, the first and last rows of the plot can be harvested by hand shortly before selection and harvesting. Similarly, the alleys can be mechanically slashed or hand harvested to facilitate moving across the field and harvesting. The second principle is to lay out the trial in a fashion that it occupies a piece of land of regular shape, because this facilitates the handling of the rest of the land by the farmer.

The management of trials residues (borders, fillers around trials, border rows and what is left of a plot after taking samples) is an important organizational issue because it is a potential source of dispute. As a general principle, as in many other organizational issues in PPB, this needs to be discussed in advance with farmers, justifying why the handling of experimental plots is different from the handling of a field planted for large-scale production, underlining the need to generate information to use later in selection, and the need for as much precision and accuracy as possible to obtain correct estimates of the genotypic values of the breeding material (the scientists do not necessarily have to use these terms when discussing with farmers!). As mentioned earlier, the guiding principle is to justify and pose the problem, and involve farmers in the process of finding the most mutually suitable solution.

9.4.7 Farmer selection

An organizational issue peculiar to participatory breeding programmes is the selec-

tion done by the farmers. This is one of the most important operations (and one that makes the breeding programme participatory). It is also one of the activities that, if done properly, can generate a strong sense of ownership, and enhance farmer skills as far as knowledge of the genetic material is concerned.

As for other organizational issues, it is impossible to give general recommendations, because the baseline can be very different in different communities. One of the extreme situations is represented by communities where there is only a vague notion that different varieties do exist, but farmers have had only sporadic contacts with scientists, and these contacts have been mostly of the type "I am here to tell you what do; you do it, and I will come back to check if you did it well!". In these communities, farmers often ignore the sexual reproduction in plants and therefore the diversity itself within a crop is surrounded by an aura of mystery. The other extreme is represented by communities who already have a solid experience in breeding and experimenting.

Most of our experience has been with the first type of situation, which is not necessarily the most difficult, but is certainly the one in which PPB takes more time to develop and one in which PPB evolves from consultative to collegial (as defined under 9.4.3). Therefore we will illustrate some general principles that we followed with the first type of situation, and how these principles need to be modified in the case of the second situation. We will consider in particular two aspects of farmers selection, namely 'when to select' and 'how to select'.

The timing of selection depends strongly on the crop and its uses, on the environment and on the traits farmers consider important. This is a typical aspect of the overall activity, and one which needs to be discussed with farmers during the planning of the programme because it has implications for the amount of time farmers need to allocate to selection and on the total number of experimental units (plots or plants) farmers can handle. It also has implications for the degree of involvement of the scientists where some of the traits that are important to the farmers need to be measured.

The choice of the ideal time for selection is highly individual: some farmers prefer to visit the field often during the cropping season, while others, particularly in unpredictable environments, claim that only shortly before harvesting is it possible to assess the real value of the breeding material. Farmers may also change their preferences in relation to both when and how to select. Farmers who were used to an organized 'selection day', whereby all the farmers assembled at a meeting point and visited and scored the various trials, subsequently demanded to do the selection by themselves on a date convenient to them. In fact, it is obvious that while the first way of organizing the selection favours exchange of ideas among the participants, it also implies fixing a date in advance that later may be no longer convenient to some participants. The second solution has the advantage of allowing many more farmers to do the selection as they are free to choose when to do it. This obviously requires that the scoring sheets be made available ahead of time.

The scoring method using by farmers during selection is another organizational issue, and like many others, the starting point can be different in different countries and in different communities within the same country. In some communities, some farmers are used to score different entities based on merit or value; in others there is no previous experience. The example of scoring

school homework is often useful. For some farmers, it is easier to use words representing different categories such as 'undesirable', 'acceptable', 'good', 'very good' and 'excellent', which later be translated into a numerical scale. With time, and particularly with those farmers participating regularly in the selection session(s), the scoring method may change, particularly when farmers within the same community use different methods, and farmers will eventually converge towards a common scoring method.

When scoring implies 'writing' (words, symbols or numbers) there is risk of excluding farmers unable to read or write. The problem can be solved by flanking the farmers who need assistance with a researcher, an extension staff member or another farmer; this requires additional organizational arrangements, particularly in remote areas. In those cases, the ideal solution is to make the communities capable of organizing themselves as much as possible.

Other methods of scoring breeding material include the identifications of the best entries with ribbons of different colours (depending on the category).

9.4.8 Visits to farmers

In a participatory breeding programme it is very important to maintain contacts with farmers beyond and besides specific scientific activities. These 'courtesy' visits are not only instrumental in building and maintain good human relationships between scientists and farmers by bridging gaps, but are an incredibly fertile reciprocal source of information. Often farmers like to converse on issues not directly related with the specific participatory programme, but related to the multitude of challenges that farmers, particularly those in marginal agricultural environments, continually face. This helps scientists to put the issue of developing new varieties of a given crop in a broader context.

9.4.9 Note taking, data management and analysis

The trials conducted in a participatory breeding programme need to generate the same quantity of information and of the same quality as those in a conventional programme, for two reasons: first, because the information has to be used to take the final decision of which material to promote and which material to discard, and, second, because the information can be later used in the phase of variety release. We learned that in addition to the visual selection, farmers may want to have access to some quantitative data to reach a final decision. This is an additional issue to discuss at the onset of the programme because if this is required by the farmers, the trials have to be organized in such a way as to allow collecting data on the traits considered important by farmers, analysing the results with appropriate statistical analysis, and reporting the results in a format that makes the information fully accessible to farmers.

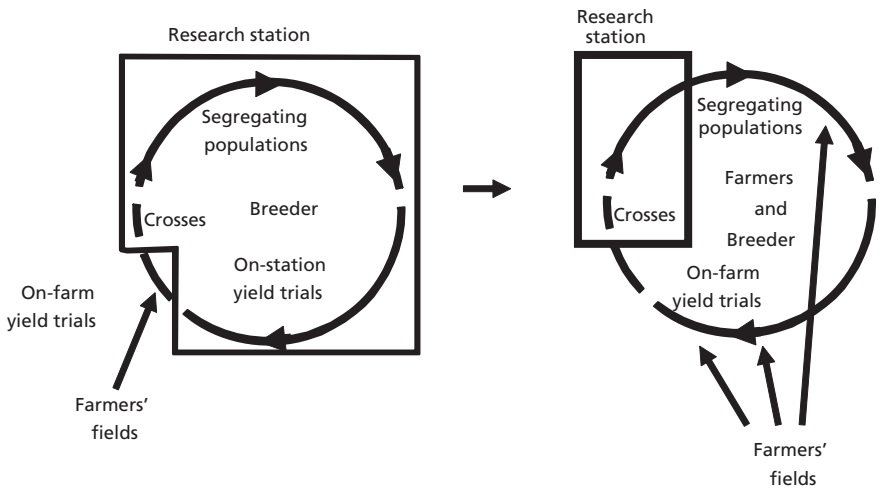
Collecting field data may go beyond the time, the facilities and the expertise of the farmers, but this is a possibility that can not be ruled out *a priori*. However, as in most similar cases, the issue needs to be discussed with the farmers so that it is becoming almost a service that the scientists provide them.

As for the analysis of data from participatory breeding trials, the reader could refer to Bellon and Reeves (2002), who present a wide range of analytical tools, and to Chapter 20 in this volume.

9.4.10 Managing the transition phase

In this section we will consider the organizational issues faced by breeders who decide to migrate from a CBP to a

FIGURE 9.2
Conventional plant breeding is a cyclic process that takes place largely within one or more research stations (left) with the breeder taking all decisions; participatory plant breeding is the same process, but takes place mostly in farmers' fields (right) and the decisions are taken jointly by farmers and breeders



decentralized and participatory breeding programme. We will not consider the case of transforming a decentralized non-participatory breeding programmes into a participatory programme because this only require solving the organizational issues associated with farmer participation.

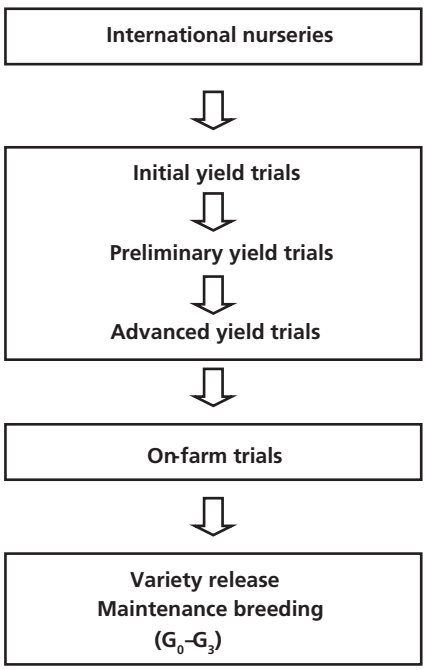
In general, the problem is to transfer a cyclic process taking place largely within one or more research stations (Figure 9.2, left) to farmers' fields (Figure 9.2, right), and to change the process of decision-making in the way discussed earlier. The general organizational issues in managing the change is that, because it is unwise to get rid of breeding material, the transfer of the programme to farmers' fields should start from the first step that the breeder intends to transfer and implies that, till the transfer is completed, the CBP and the DPBP will coexist. This should be clearer from the examples given later.

We will discuss two scenarios, which are the most common in breeding programmes in developing countries. The first scenario concerns breeding programmes that do not generate genetic variability, but in which the base germplasm is introduced from other institutions (generally international breeding programmes) and sometimes include locally collected germplasm and wild relatives. The second scenario is fully-fledged breeding programmes with all the steps described in Chapter 3, and which may include acquisition through germplasm collection and molecular breeding.

First scenario: breeding programmes with only the selection and testing stages

We will examine the organizational issues in managing the transition of a plant breeding such as the one shown in Figure 9.3, which represents a situation common to several countries. In such a breeding programme,

FIGURE 9.3
Schematic representation of conventional plant breeding that relies on introduced breeding material as the source of genetic variability



All the phases before the on-farm trials are conducted on a research station.

the first step (as defined in Chapter 3) is replaced or represented by the introduction of breeding material (including segregating populations, nurseries and yield trials) from other breeding programmes, usually from international organizations. The incoming breeding material is grown on station for an initial cycle of selection (mostly negative selection), followed by a series of yield trials conducted in a number of research stations for a number of years. The yield trials have different names (we will use initial, preliminary and advanced), and most typically are conducted over a period of three or more years: during this period the number of entries decreases and the plot size increases.

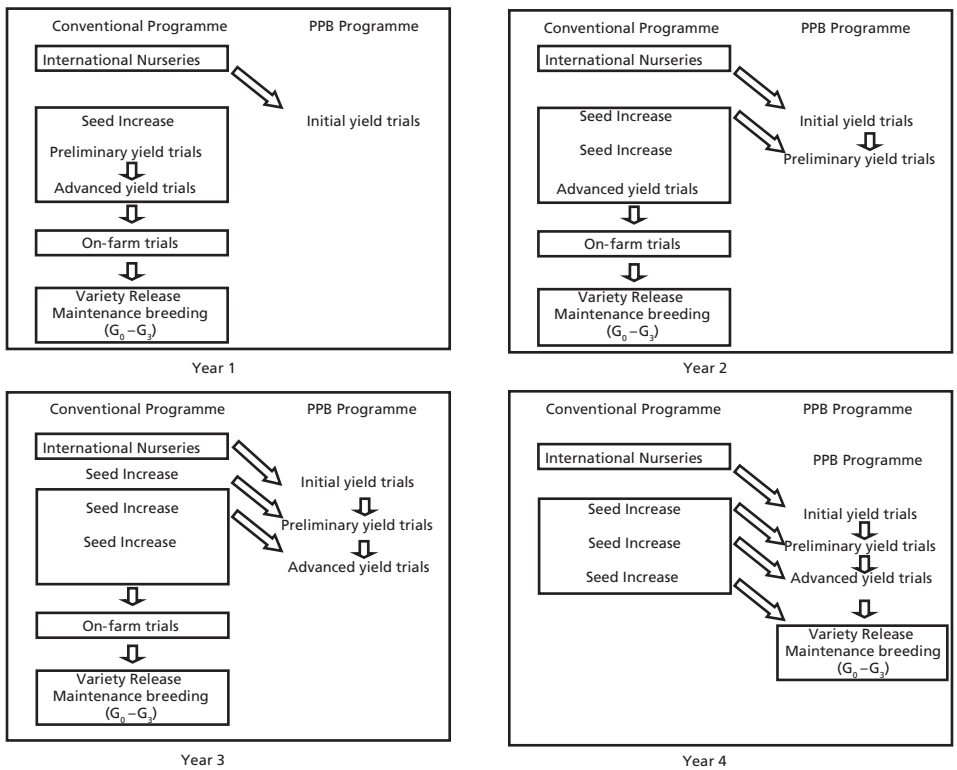
At the end of the three or more years of on-station testing, the entries considered as promising are tested in on-farm trials, which are usually repeated for two or three years and generate the data used, together with those obtained on station, to support the submission of a variety for release. There are cases in which the on-farm trials, or at least some of them, are also conducted on station.

The possible steps to modify such a programme are shown, year by year, in Figure 9.4. The process of modification begins with planting the initial yield trials in farmer's fields (where and how many is based on what has been discussed earlier in this chapter) rather than on station. Therefore, in the first year of the participatory programme, all the nurseries and trials will be as in the conventional system, except the initial yield trials, which will be planted in farmers' fields. The remnant seed of the initial yield trials is planted in a research station with reliable rainfall or irrigation facilities for seed increase.

In the second year, the preliminary yield trials, containing the entries selected by the farmers in the various locations, will be planted at the same sites using the seed produced on station. Using a common seed source is important to avoid biased comparisons between entries selected in different locations. Also, a new set of initial yield trials will be planted in farmer's fields. On station, together with the advanced yield trials of the conventional programme, the seed increase of the breeding material tested in both the initial and the preliminary yield trials will be conducted.

In the third year, the advanced yield trials, containing the entries selected in the preliminary yield trials by the farmers in the various locations, will be planted at the same sites using the seed produced on

FIGURE 9.4
Steps to modify a conventional plant breeding programme that relies on introduced breeding material as the source of genetic variability into a decentralized-participatory breeding programme



In each box the conventional program is on the left and the participatory on the right

station; therefore, in the third year, all the three categories of trials will have migrated into the PPB programme, while only seed multiplication is conducted on station.

In the fourth year there is no more need to plant the 'on-farm trials' because all the trials have already been conducted on farm, and if the data are considered sufficient, and there is material worth releasing, the procedure for variety release can be initiated, while the promising lines are further multiplied.

A number of activities can be conducted on station in parallel with the participatory programme. For example, the incoming breeding material can be tested for important pests and diseases, while multiplying the seed for the initial yield trials, as the screening can continue in suitable locations (plastic houses, hot-spot sites) during all testing and selection stages, using part of the seed kept for increases.

Also, the lines from the advanced yield trials that are candidates for release can be used as parent in a crossing programme. As we are discussing the case of a breeding programme without crossing programme, these lines could be sent to the institution(s) supplying the incoming breeding material with a request that they use them in

targeted crosses. Incidentally, this is a case of PPB in which the partners are two breeding programmes, one national and one international.

Links can be also easily established with the activities of one or more genebanks, where they exists For example, a genebank could conduct a preliminary evaluation of new germplasm (locally collected or acquired from other genebanks), involving farmers in the assessment. The selected accessions, after one further cycle of seed multiplication, can then pass into the PPB programme in the initial yield trials. The information collected on the material coming from the genebank should be shared with the genebank, becoming part of the Passport data.

Second scenario: fully-fledged breeding programmes (self-pollinated crops)

The migration of a fully-fledged CBP is more difficult to generalize because of the multitude of methods used to handle the segregating populations, even within crops characterized by the same mating system. Therefore, we will examine the case of some of the most commonly used breeding methods, illustrating how they can be changed into a participatory programme.

Figure 9.5 illustrates a typical example of a breeding programme of a self-pollinated crop, based on a classic pedigree method. This is still fairly widespread in several developed countries, and can be easily transformed into a participatory programme, by moving the on-station yield

FIGURE 9.5
Schematic representation of a conventional plant breeding programme of a self-pollinated crop based on a classical pedigree method (left): all the phases before the On-farm Verification trials are conducted on research station. On the right, the same programme conducted in a participatory mode

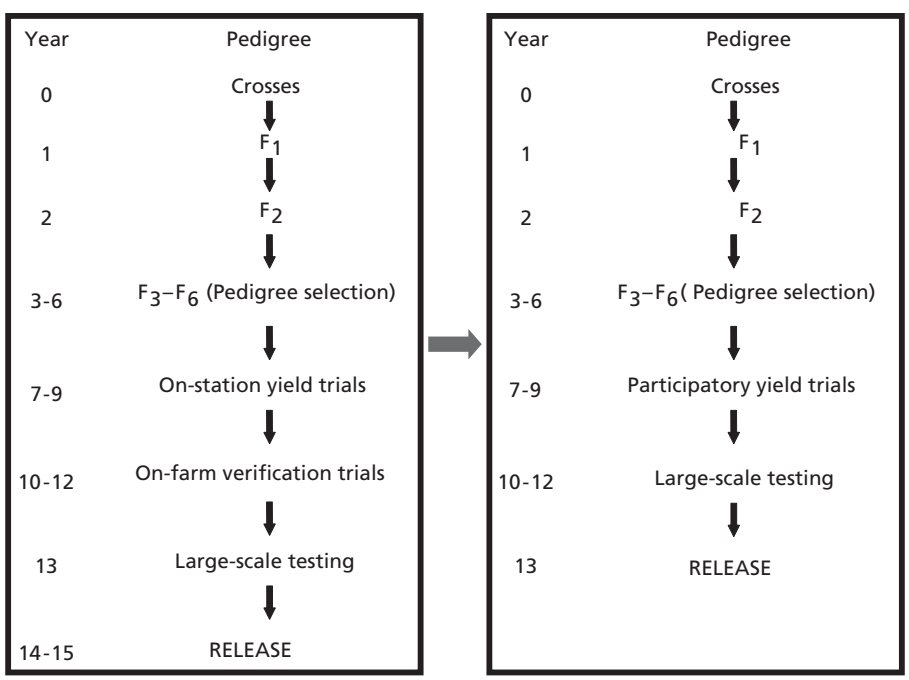
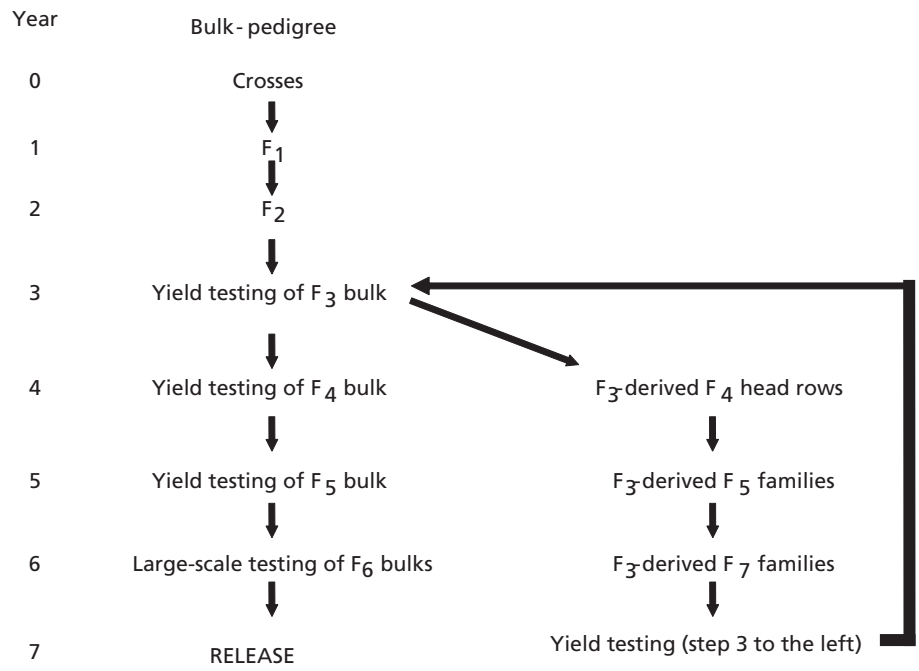


FIGURE 9.6
Schematic representation of a participatory plant breeding programme of a self-pollinated crop based on a bulk-pedigree method that allows selection between crosses in farmers' fields through yield testing in three successive cropping seasons (left) and selection within selected crosses on station (right)



The pure lines derived from selection within populations will be yield tested through steps 3 to 6.

trials onto farmers' fields in a phased fashion, as shown in Figure 9.4. This will make the 'on-farm verification trials' redundant, so that the change will shorten the period before release by at least three years, and the choice of the candidates for release can be based on farmers' preferences rather than on agronomic performance alone.

The migration from the classical on-station pedigree method on the left of Figure 9.5 to the equivalent programme conducted in a participatory mode (Figure 9.5, right) takes place through the steps shown in Figure 9.4.

One alternative breeding method that allows selection, both between and within crosses, to be conducted in a participatory mode is the bulk-pedigree method shown in Figure 9.6.

The method, described in detail by Ceccarelli and Grando (2007), is based on the yield testing in farmers' fields of early segregating populations (F_3 bulks). The selected bulks are yield tested as F_4 bulks for a second year, and those that are selected are tested for a third year as F_5 bulks. In parallel to the yield testing of the populations (selection within crosses), it is possible to conduct on-station pure-line selection within the selected populations (left) by collecting heads of the selected F_3 bulks. The F_3-derived F_4 head rows are

promoted to the F_5 only if farmers select the corresponding F_4 bulks. The process is repeated in the F_5 and the resulting families, after one generation of increase, return as F_7 in the yield-testing phase. Therefore, when the model is fully implemented, the breeding material that is yield tested includes new bulks as well as pure lines extracted from the best bulks of the previous cycle.

The method has a number of advantages: (i) the participation of farmers can be introduced very early in the overall process. The method can actually start with the F_2 if the amount of seed available is sufficient; (ii) during the pure-line selection, it is possible to screen for biotic stresses, quality traits or other traits important to farmers and with high heritability, using conventional or molecular approaches; and (iii) the method can also be used solely for selection between crosses in those cases where the system of variety release is not too strict in terms of uniformity.

While the aspects of managing the transition phase have been discussed with specific reference to self-pollinated crops, the concepts underlying the process are equally applicable to cross-pollinated crops.

9.4.11 Institutionalization of participatory plant breeding

Institutionalizing PPB (i.e. mainstreaming and scaling-up) must be one of the main objectives when setting up a participatory breeding programme. This is because it is very unlikely that individual, small-scale PPB projects, even though very successful at local level, will ever determine impact at national level in terms of production increase, for example, even if this is not the only impact expected from a PPB programme. At the same time, only collaboration between the institutions that have responsibility for plant breeding and farmers could exploit the relative advantages of the two partners, i.e. the extraordinary ability of institutions to generate variability, and their continuity, versus the extraordinary ability of farmers to extract what can improve their livelihood from that variability under their conditions.

We have already given an example of the technical aspects of institutionalizing in the section *Managing the transition phase*. However, the major issue with the institutionalization of PPB is to make this method acceptable to national and international research institutes as being the way in which plant breeding is conducted by the institute.

Unfortunately, the several cases of both successful and unsuccessful institutionalization of PPB do not allow drawing a general lesson or a general methodology on how to obtain institutional recognition of PPB as an approach that effectively combines the development of improved varieties with development objectives aiming at alleviating rural poverty and improving local food production (Almekinders and Hardon, 2006).

One good example to illustrate how difficult it is to understand what influences policy and managers of agricultural research is the experience at ICARDA based on the work done in nine countries (Algeria, Egypt, Eritrea, Iran, Jordan, Morocco, the Syrian Arab Republic, Tunisia and Yemen) with a number of crops, with one in common (barley), by the same team of scientists with a similar methodology. This work yielded contrasting results in terms of institutionalization, ranging from institutional rejection, as in the Syrian Arab Republic and Egypt, where the programme continues as a direct ICARDA-farmers collaboration, and Tunisia, where, at the end of a special project, all the activities ended, to full insti-

TABLE 9.1
Status of nine PPB programmes conducted by the same Institution (ICARDA) in nine different countries and date of Institutionalization

Country	Crop(s)	Date started	Date ended	Date of institutionalization
Syrian Arab Republic	B	1995/96	Continuing	N/A
Morocco	B	1996/97	Continuing	2000 (see text)
Tunisia	B	1996/97	1999	N/A
Yemen	B, L	1999	2002	2003 (see text)
Jordan	B, C, BW, DW	2000	Continuing	2005
Egypt	B	2006	Continuing	N/A
Eritrea	B, L, C, F, BW, DW	1998	Continuing	1998 (see text)
Algeria	B, DW	2006	Continuing	2007
Iran	B, BW	2007	Continuing	2008 (see text)

Key to crops: B= barley; L = lentil, C = chickpea, F = faba bean, DW = durum wheat, BW = bread wheat.
Date is that at which the programme was fully supported by Government institutions either financially or ideologically (see text for details). N/A indicates not institutionalized.

tutional acceptance of the methodology, as in Algeria, Eritrea, Iran, Jordan, Morocco and Yemen even though with different timing and modality (Table 9.1).

In the Syrian Arab Republic and Egypt, national institutions were actually involved, but in the case of Syria, the collaboration was terminated, and in the case of Egypt, the institution involved is not the one having the mandate for plant breeding.

An intermediate institutionalization was observed in Morocco, where the National Barley Breeding Programme at INRA at the end of a project (the same project involving Tunisia) adopted PPB in the programme for dry areas only.

In Yemen, a two-year project has had the power of introducing the concept of participation in all the research activities of the institution (AREA), while in Jordan only the breeding programmes have been gradually transformed by NCARE into PPB programmes (the transformation is still in progress). In Jordan, as well as in Algeria, PPB is now taught at the Universities by the same national scientists who are implementing the programme in the field.

Eritrea and Iran are special cases. Eritrea, being a recently independent country, did not have an existing research programme, and therefore when ICARDA started its collaboration with the country, it was with participatory methodology involving both the Ministry of Agriculture and the University. In Iran, in contrast, one of the two leading institutions (DARI) involved in plant breeding was in favour of experimenting with PPB, while the other (SPII) was opposed. Therefore, in the area for which SPII is responsible, the current programme is conducted as an NGO-Farmers-ICARDA collaboration, with germplasm provided by ICARDA, while in the area for which DARI is responsible we have full institutional support, with provision of germplasm and latterly with full financial support of the programme by the Provincial Agricultural Office.

The different reactions of the Institutions are not easy to explain. For example, in the Syrian Arab Republic, after nearly 15 years during which we conducted travelling workshops for policy-makers (including members of the variety release committee), research managers and scientists, several training courses for scientists, the publication of two scientific papers in refereed journals with national scientists as co-authors, several

seminars and university lectures, and more importantly the adoption by farmers of a number of varieties (not even considered for release) with large production increases, there is still institutional opposition to even consider PPB as a complementary approach to conventional breeding.

In contrast, and after only two years and with no travelling workshop, no training, no scientific papers, and only a few seminars and meetings, there has been a complete uptake of the methodology by at least one institution in Iran and a very similar reaction was observed from the two leading institutions in Algeria (INRA and ITGC) after only three years.

The facts that one crop is common to all cases, that in those countries with contrasting institutional reactions the crop is used mostly as animal feed, and that the farmers belong to the same culture, make it even more difficult to interpret the different attitudes of policy-makers and scientists.

It appears as if ultimately the success in institutionalizing PPB depends on individuals, on their attitude to innovations and on the power relationships within the institution. In our experience, the institutionalization has been enormously facilitated by the presence of a person within an institution with sufficient moral authority to influence all the others.

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CHAPTER 10

Selection methods. Part 2: Pedigree method

Flavio Capettini

10.1 INTRODUCTION

Since the beginnings of agriculture more than 10 000 years ago, crop producers have been striving to obtain better crops. Different procedures have been followed to reach their objectives, with various results. Modern plant breeders, after the re-discovery of Mendel's laws just over a century ago, tried to introduce new knowledge in the process, obtaining sizeable progress in a relatively short period of time. Although different formal methods have been described, there is the general impression that modifications are more the common rule in breeding programmes rather than the strict methods described in books. Ultimately, every applied breeder has their own approach and priorities to reach their objectives, using a package of breeding tools, available knowledge and resources. This package should be matched to the realities of their socio-physical environment, which include the mechanics of institute where the work is carried out, the crop, the agro-ecological target area, and the socio-economic parameters of the target farmer group. Besides all the above, resource availability is probably becoming more and more the most important factor in determining the size and methodology of a breeding programme, for both public and private entities. Obviously, revenue expectations also play a decisive role in the size of investment that is applied to a programme.

10.2 PEDIGREE METHOD

The Pedigree Method—or to be more precise: the Modified Pedigree Method—is probably the most popular protocol used by plant breeders to advance generations during the inbreeding process in self- and cross-pollinated species, aiming to obtain desirable homozygous lines. Although there are as many modifications as breeders available, the general principle is what gives the method its efficacy: the genetic value of an individual can only be proven by the performance of its offspring. This method relies strongly on the maintenance of records of the parent-progeny relationships. Selection is usually performed on visual (or laboratory, marker assisted selection (MAS), etc.) assessment of significant, highly heritable traits. Many reasons drive breeders to combine pedigree with mass or bulk methods (Table 10.1).

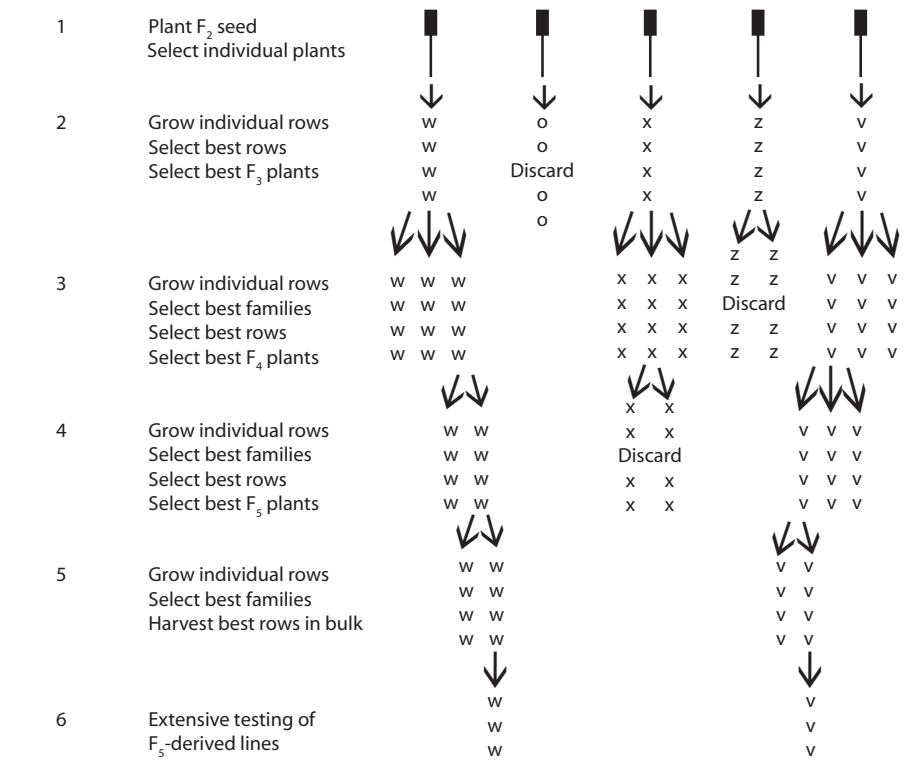
TABLE 10.1
Example of a programme following the Pedigree Method

Growing Cycle	Procedure
1	Plant 250 – 5000 F_2 plants.
	Select individual plants (300)
2	Grow $F_{2:3}$ rows (300)
	Select best rows (50)
	Select best plants in rows (2–3)
3	Grow $F_{3:4}$ lines (150)
	Select best families (25)
	Select best rows in families (3)
	Select best plants in rows (2)
4	Grow $F_{4:5}$ lines (80)
	Select best families (15)
	Select best rows in families (2–3)
	Select best plants in rows (1)
5	Grow $F_{5:6}$ lines (20)
	Select best families (15)
	Harvest best rows in bulk
6	Testing of $F_{5:7}$ lines in two
	locations, one rep per loc.
7	Multi-location
	testing begins ($F_{5:8}$).
	Harvest heads from yield plots in one replication to seed purification.
8	Multi-location testing.
	Grow 50-100 $F_{8:9}$ head-rows of lines still in test to produce pure seed.

10.2.1 Origin

The term 'pedigree selection method' was applied when first used by Svalöf in Sweden

FIGURE 10.1
Diagrammatic representation of the pedigree method



Courtesy of Fehr, 1987a

in 1891, where single plants were selected from an existing cultivar or a landrace population. Newman (1912) described how selected plants were laid out in the field as plant-row, ear-row or head-row plots. The Vilmorin Company in France developed the system independently, where it was termed the 'Vilmorin method of selection' (Fehr, 1987a).

10.2.2 Implementation

The method can be initiated or stopped at any generation during the inbreeding process. When combined with mass or bulk selection, it is generally applied in generations closer to when homozygote lines are developed. This is because other methods are less labour (therefore resource) intensive and easier to apply in earlier generations. Generally, selection starts with an F_2 population and goes on until homogeneous lines are developed. In the next cycle, the best $F_{2:3}$ lines are selected, followed by the selection of the desirable F_3 plants within each line. In all subsequent generations selection is performed on the most desirable families first, followed by the most desirable lines within that family, to finally select the best plants within each line, which are harvested individually (Figure 10.1).

TABLE 10.2
Expected additive genetic variability among and within lines during inbreeding process without any selection

Generation of lines	Additive Genetic Variability	
	Among Lines	Within Lines
$F_{2:3}$	1	1/2
$F_{3:4}$	1.5	1/4
$F_{4:5}$	1.75	1/8
$F_{5:6}$	1.875	1/16

Although breeding is a 'numbers game' – the greater the numbers managed, the higher the probability of finding the right or a better combination of genes – the size of the programme will always be limited by the resources available. This would include the level of the breeder's and support personnel expertise the institution is able to hire, land, hardware, technology and time. At the beginning of each season, the breeder should decide how many progeny rows they will be able to grow for all selection generations.

The pedigree method also relies heavily on the expected genetic variability in each generation, and that will determine the number of selections made (Table 10.2) (Fehr, 1987a). The genetic variability expected within each line is at a maximum at the $F_{2:3}$ generation, decreasing by half in each following generation.

10.2.3 Genetic background

The most important factor to be considered is the genetic variability present among and within lines during the inbreeding process (Table 10.1). Additive epistasis is generally much smaller than the additive portion of total genetic variability. Variability associated with dominance and dominant epistasis cannot be utilized in inbred lines. Dominance can complicate the selection of homogeneous and homozygous lines by not allowing differentiation of heterozygous individuals from dominant homozygous. The same can occur with the heterosis expressed by heterozygous individuals, slowing the homozygosity process (Fehr, 1987a).

10.3 PROS AND CONS OF THE PEDIGREE METHOD

10.3.1 Advantages of the pedigree method

- The 'art of breeding' can be extensively exercised. The breeder can effectively decide in the field the shape of the breeding programme and see the effect in every generation. Inferior genotypes can be discarded early in the process, allowing the use of higher volumes for early generations in the programme, and retaining only the good ones for the later, expensive, replicated experiments stage.
- Different locations and environments can be used in each growing cycle, allowing selection for different traits not expressed everywhere. Populations can be replicated in hot spots, i.e. environments where the desired selection traits are expressed at a maximum, to increase the probability of selecting for specific traits.
- The breeder can manage the amount of generic variance they want to keep within and between families, as well as the number of families.
- Different qualitative and quantitative traits can be selected at the same time in different generations, including traits being expressed at plant as well as grain level.

10.3.2 Disadvantages of the pedigree method

- Cannot be utilized in environments where genetic variability for the characters of interest is not expressed. If one cannot use off-season nurseries, there will be an associated increase in the length of time

for cultivar development compared with other methods of breeding.
- Considerable record keeping.
- An experienced person must do the selection (at least we flatter ourselves in thinking so).
- Requires more land and labour than other methods of inbreeding.

Besides the points above, there are questions concerning applied breeding programmes, especially those in the private sector. Questions about why the extensive data recording is needed, if the plants that are kept in the programme are there because they showed superiority anyway. Pedigree becomes difficult to use for quantitative traits, especially those with lower heritability. Despite the named disadvantages, that are more due to the different circumstances and priorities, this is the method that resulted in significant increases in the genetic gain in breeding programmes early in the twentieth century. The criteria would depend on how narrow the cross is, the heritability of the traits, costs of the evaluation, worth of the trait and resources available. Modifications were carried out once the system was understood, and it helped to acquire deeper knowledge of the high number of genes acting in plant development.

10.4 SINGLE-SEED DESCENT METHOD

Single-seed descent is a method to rapidly advance generations of inbreeding populations before starting the evaluation of individual lines, which is frequently used in conjunction with the pedigree method. The concept was proposed by Goulden in 1941. He noted that a breeding programme can be divided into two stages: the development of pure lines from segregating populations; and selection of desired pure lines from among those produced. The disadvantage of the pedigree method was that only one generation could be grown each year. By separating inbreeding from selection, the first process could be accelerated until homozygosity was reached. Working with wheat, he suggested that the number of progeny to be grown from a plant be one or two, growing two generations in the greenhouse in each autumn and winter, and one generation in the field during the summer. With this method, the F_6 generation can be reached in two years, compared with the five years needed with the Pedigree Method. Once homozygosity is achieved, a large number of lines can be tested for the desired traits.

The harvest of one seed from each plant during inbreeding was first described by Johnson and Bernard in 1962 for soybeans, and the first time this method was termed single-seed descent was by Brim (1966), who considered it to be a modified pedigree method.

The usual procedure is to harvest a single seed from each plant in a population, bulk the harvested seeds and plant the bulk in the next generation. The procedure can be started in the F_2 and continued until the desired level of homozygosity is achieved. The number of plants that will be needed in the last generation of inbreeding should be decided and the number of initial plants should be calculated backwards to the F_2 generation, taking into account the expected losses due to lack of germination.

In his book, Fehr (1987b) also describes modifications of the single-seed descent method, such as a multiple-seed procedure and a single-hill procedure. The objectives are the same: to obtain rapid generation advance. The methods are well suited for use in greenhouses and winter nurseries, where genotypes perform differently from their area of adaptation.

10.5.1 Advantages of the single-seed descent procedures

- They are easy to manage and speed up the inbreeding process as no special laboratories or techniques are needed in comparison with other methods, e.g. double haploid production.
- Procedures are well suited for environments where otherwise only one generation per year can be grown in the field.
- Can be less expensive than field selection where the costs of land and land management are high.

10.5.2 Disadvantages

- The size of the population should be adjusted for germination losses.
- All the F2 plants may not be present in the line evaluation due to germination losses, decreasing the genetic variance.
- The amount of seed available at the end of the inbreeding process is reduced, needing additional growing cycles just to multiply seed, thus delaying the total process.

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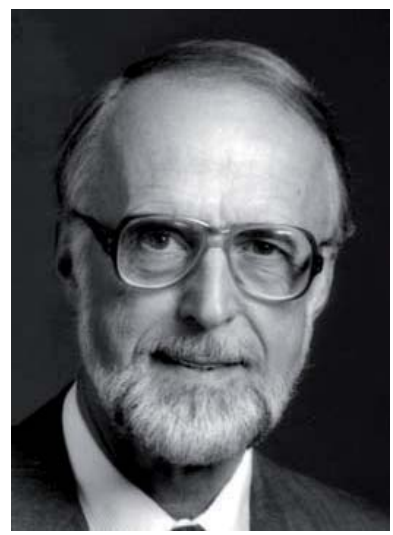
CHAPTER 11

Selection methods Part 3: Hybrid breeding

Donald N. Duvick*



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11.1 INTRODUCTION

When science-based plant breeding began at the beginning of the twentieth century, breeders at first produced only pure-line cultivars in self-pollinated species such as wheat (*Triticum aestivum* L.), or open-pollinated cultivars in cross-pollinated species such as sugar beet (*Beta vulgaris* L.). But following the success of hybrid maize (*Zea mays* L.) in the United States of America in the 1930s, breeders of field and horticultural crops began to look for ways to breed and produce hybrid seed of their own crops – crops other than maize. They usually intended to use hybrids to harness heterosis (see Chapter 2) and thereby produce higher yielding cultivars, but they also saw a potential for improving non-yield traits with more precision and breadth than could be achieved in either pure-line or open-pollinated cultivars. Additionally, seed companies saw commercial possibilities in hybrids. Growers would need to purchase hybrid seed every year because saved seed of a hybrid (F_2 seed) is significantly lower yielding than the hybrid parent, and it also may lack uniformity for important traits should such uniformity be necessary.

11.2 ORIGINS OF HYBRID BREEDING: CROP-SPECIFIC TECHNOLOGIES

11.2.1 Maize

Hybrid maize, made by crossing inbred lines of maize, was introduced to farmers in the United States of America in the late 1920s and early 1930s (Crabb, 1993; Jenkins, 1978). Maize breeders from both public and private sectors developed the first inbred lines and hybrid cultivars. Private seed companies produced and sold seed of the best hybrids. American farmers rapidly abandoned their open pollinated cultivars (OPCs) in favour of hybrids, even though they had to buy new seed every season (USDA-NASS, 2000). The best hybrids yielded more than the best OPCs, although not a great deal more, about +15 percent (e.g. Iowa State Department of Agriculture, 1934) . They also had stronger roots and greater resistance to stalk rot and subsequent stalk lodging. The farmers could easily distinguish the hybrids' advantages in standability.

North American farmers had begun to adopt mechanical maize harvesters ('corn pickers') on a large scale in the 1920s and 1930s; these farmers were attracted to maize hybrids because of the improvements in standability. Fewer lodged plants meant fewer ears missed by the corn picker, and therefore harvestable yield was increased significantly.

Two years of disastrous drought (1934 and 1936) in the United States Corn Belt gave further impetus to adoption of maize hybrids; the best hybrids were much more heat and drought tolerant than the OPCs. The author's personal experience is that, in 1936, on our home farm in Illinois, the advantage of hybrid over local OPC was so great that the high school vocational agriculture instructor brought his class out to see this on-farm demonstration of the superior drought tolerance of hybrid maize (then a new and rather mysterious crop). Our OPC was essentially 100 percent barren and was used only for fodder; the hybrid made enough grain to warrant harvesting. There was a concomitant effect in the seed trade.

A common statement in those days was that every dollar spent on hybrid seed should produce extra grain yield worth at least three dollars. Fortuitously in the 1930s, the federal government instituted measures to support the price of maize grain. This gave farmers confidence that they could get a fair return for their extra grain yield. Before support prices were

> *Hybrid 307 was the double cross that I would say really put us in the seed-corn business. A lot of farmers knew about hybrid corn by that time and some of them were piddling around and trying a little bit of this and a little bit of that. Most of them waited to see what happened; they didn't think it would really amount to anything. We put out some of the hybrid in 1936 and it was so drought resistant that it convinced a lot of people that hybrid corn was something they wanted to grow.*
>
> (R. Baker, pers. comm., 1990)

instituted, maize grain prices (in the midst of the Great Depression of the 1930s) were so low that farmers might have decided against spending money on hybrid seed despite its extra yield, reasoning that the return on investment in hybrid seed might not be realized.

Seed production of hybrid maize was relatively simple because of the separation of male and female flowers: male in the tassel and female in the ear. Rows of detasselled plants could be used as seed parents ("female") to be pollinated by adjacent rows of non-detasselled plants ("male") of a different genotype. No other crop had such phenotypic advantages; breeders of other species therefore knew they had to look for other ways to produce hybrid seed efficiently and economically.

11.2.2 Horticultural crops

Genetic investigations in horticultural crops suggested several methods with the potential to make hybrid seed economically, at prices farmers could afford (reviewed in Duvick, 1966).

Cytoplasmic-nuclear male sterility was discovered in crops such as onion (*Allium cepa* L.) and beets (*Beta vulgaris* L.), and breeders of those crops soon developed hybrid cultivars. Sterility resulted from interactions between a specific cytoplasmic genotype and a specific nuclear genotype. Plants with sterile cytoplasm plus nuclear non-restorer genes were male sterile; plants with sterile cytoplasm plus nuclear restorer genes produced fertile pollen (fertility restoration). In contrast to plants with sterile cytoplasm, plants with normal cytoplasm were male fertile when they carried either of the nuclear genes: restorer or non-restorer. Fertility restoration was not needed for onion and beet hybrids, grown for their vegetative parts, but it was needed when cytoplasmic male sterility was used to make hybrids of crops grown for fruit production.

Hybrid seed production required efficient methods of cross-pollination to keep costs of production (and thereby seed prices for the farmer) at economically low levels. Bees did the pollination for onions, and wind served for beets.

Self-incompatibility, present in several species of horticultural crops such as broccoli and cabbage (*Brassica oleracea* L.) was used to make seed of hybrid cultivars. The term 'self-incompatibility' as used here means the inability of a plant to set seed when self pollinated, even though it can form normal zygotes when cross-pollinated, and its pollen can fertilize other plants. Breeders used two types of self-incompatibility: gametic and sporophytic, depending on which kind prevailed in a given crop species. Nuclear genes govern both types, and genetic interactions can be complicated but manageable for development of lines to be used as seed parents or pollinators.

Hand emasculation followed by hand pollination was (and still is) used to make hybrid seed of some horticultural crops, for

example eggplant (*Solanum melongena* L.) and pepper (*Capsicum annuum* L.). Despite high inputs of labour, it was used successfully to make hybrid seed of crops with high value, relatively low seed requirements, and relatively abundant seed numbers per pollinated flower. Hybridization via hand emasculation was often performed in developing countries with relatively low labour costs, to lower the cost of seed production.

Genetic male sterility was used to make hybrid seed of a few crops (e.g. pepper), although, like hand emasculation, its use required considerable inputs of hand labour. Segregating populations of heterozygous lines could be grown as female; pollen-fertile plants would be removed at the time of flowering, or earlier if they could be identified with a linked marker gene.

Monoecious (male and female organs on the same plant but in different flowers), *gynoecious* (plants with only female flowers) *or andromonoecious* (plants with both bisexual and male flowers) *genotypes*, common in some families such as the melons (Cucurbitaceae), were manipulated to produce hybrid seed, using bees as pollinators, or sometimes with hand pollination (made easier because hand emasculation is not required). An example in cucumber: a gynoecious inbred used as female might be crossed to a monoecious inbred used as male, with bees as pollinators (Janick, 1998). Chemicals can be used as hybridizing aids in some species. "Ethylene-producing compounds (Ethrel), sprayed on squash plants, turn a monoecious plant into a gynoecious plant by turning all flowers into female." (R. Heisey, pers. comm., 2005)

In summary: a variety of methods have been developed and are now employed to produce seed of hybrid cultivars of vegetable crops. Choice of a specific method depends on the species and how it is utilized. Growers of horticultural crops in industrialized nations have adopted hybrids extensively. For example, in North America in 1996, 21 of 24 vegetable crops were grown as hybrids, to varying extents (Janick, 1998). The proportion of crop area planted to hybrids varied by crop; at the two extremes, celery (*Apium graveolens* L.), lettuce (*Lactuca sativa* L.) and radish (*Raphanus sativus* L.) were 0 percent hybrid, whereas broccoli and Brussels sprouts (*Brassica oleracea* L.), pickling cucumber (*Cucumis sativus* L.), muskmelon (*Cucumis melo* L.) in the Eastern United States of America, and spinach (*Spinacea oleracea* L.) were 100 percent hybrid. Hybrid use in horticultural crops has expanded more slowly in the developing countries than in the industrialized countries but in recent years the area planted to hybrid vegetable cultivars of several species has expanded markedly in a number of developing countries, for example in countries of Southeast Asia (Kunz, 2002). In developed countries, probably more than 90 percent of the tomato and pepper seed sold is hybrid. In developing countries, the shift is to hybrid varieties. (R. Heisey, pers. comm., 2005). Yield gains can be dramatic when good quality seed of hybrids adapted to a developing country become available (M. Jahn, pers. comm., 2005).

11.2.3 Field crops

Farm production of field crops such as sugar beet, rye (*Secale cereale* L.), wheat, barley (*Hordeum vulgare* L.) sunflower (*Helianthus annuus* L.), and grain sorghum (*Sorghum bicolor* (L.) Moench.) requires large amounts of seed for planting the crop and return per unit area is relatively low compared to most horticultural crops, so expensive labour-intensive methods of producing hybrid seed (such as hand emascula-

tion) were ruled out. Breeders of field crops sought to develop hybridization techniques in which seed parents and pollinators could be grown on a field scale, and therefore looked to cytoplasmic male sterility and, to a lesser extent, to self-incompatibility or other genetic systems that could be used on a large scale. Pollination also needed to be on a field scale, such as with wind or with bees. Successful hybridization via wind pollination required that males shed abundant amounts of pollen and female lines be receptive (e.g. florets should open at appropriate times). Finally, fertility restoration in the final hybrid as grown by farmers was essential for grain crops such as sorghum, sunflower, wheat or barley.

Cytoplasmic-nuclear male sterility was used successfully to produce hybrid cultivars of several field crops, starting with crops such as grain sorghum and sugar beet, and followed by (among others) rye, pearl millet (*Pennisetum glaucum* (L.) R. Br.), sunflower, canola (*Brassica napus* L. and *Brassica rapa* L.), and rice (*Oryza sativa* L.) (Axtell *et al.*, 1999; Brown, 1999; Canola Advantage, 2005; Duvick, 1966; Geiger and Miedaner, 1999; Miller, 1987; Owen, 1945; Renard *et al.*, 1998; Stephens and Holland, 1954; Virmani, 1994; Yuan, Yang and Yang, 1994). Cytoplasmic male sterility was also used on a large scale to make maize hybrids in the United States of America in the 1950s and 1960s, but its use was greatly curtailed following the 1970 epidemic of race T of Southern corn leaf blight (*Bipolaris maydis* (Nisikado & Miyake)) (NRC, 1972; Tatum, 1971).

Hybrid wheat has been bred and produced via cytoplasmic male sterility or with chemical hybridizing agents, application of which causes pollen sterility in a line intended as seed parent in a seed production field. The resulting hybrids are male fertile. It has shown limited success, in part because of low and highly unpredictable seed yields in hybrid seed production fields and in part because heterosis within a given class of wheat contributed unacceptably small yield gains in relation to cost and reliability of seed production (Jordaan *et al.*, 1999; Knudson and Ruttan, 1988; Koemel *et al.*, 2004). Nevertheless, hybrid wheat, produced via cytoplasmic male sterility or chemical hybridizing agents, has been successful in some regions of the world (Nicolas, 2005; Saaten-Union, 2005).

> *We … have a quite successful hybrid wheat programme today in South Africa based on [cytoplasmic male sterility]. The hybrids do better than varietals in stress environments and yields are acceptable …*
>
> (T. Crosbie, pers. comm., 2005)

As with horticultural crops, industrialized countries led the way in adoption of hybrid field crops and a number of the developing countries followed closely behind them. Crops such as grain sorghum, maize[1], pearl millet, sunflower and rice are now widely grown as hybrids in many of the developing countries (e.g. Axtell *et al.*, 1999; Ejeta, 1988; Guohui and Longping, 2003; Joshi *et al.*, 2005; Swastika *et al.*, 2004; Virmani, Mao and Hardy, 2003).

Rice hybrids made with cytoplasmic-nuclear male sterility have been grown for several years in both developed and developing nations. China led the way; IRRI was influential in extending the technology to tropical Asia (Virmani and

[1] In Asian countries, excluding … China approximately 40 percent of [maize] is planted with hybrids. In Asian countries including … China approximately 76 percent of [maize] is planted with hybrids. (T. Kunta, pers. comm., 2005)

Many countries plant a high percentage of [sorghum] hybrids. In the USA, Mexico, South America, Australia and South Africa the percentage of hybrids is close to 100 percent. In India's rainy season it is close to 80–90 percent hybrid. In the post-rainy [season] they plant an OPV … almost 100 percent of which is used for local consumption … Only in many sorghum growing areas of Africa have hybrids not taken off … .

(K. Porter, pers. comm., 2005).

In Asian countries, excluding … China, approximately 40 percent of [maize] is planted with hybrids. In Asian countries including … China approximately 76 percent of [maize] is planted with hybrids.

(T. Kunta, pers. comm., 2005)

Hybrid cotton makes up about 15 percent of the Chinese acreage and 45 percent of the acreage in India. … The hybrid cotton in China appears to be increasing. A small amount of F_1 intraspecific and interspecific hybrids are grown in the USA. … The hybrids are produced by hand, probably in India and marketed by an Israeli company.

(W. Meredith, pers. comm., 2005)

Kumar, 2004). In recent years a special kind of genetic male sterility has been used as well, to make 'two-line' hybrid rice (Guohui and Longping, 2003; Yuan, 1998). Seed is produced on female inbred lines that are homozygous for environmentally sensitive recessive male sterility genes. Seed production fields are planted in an environment (e.g. long day and/or high temperature) that enables expression of the male sterility gene and thus enables successful seed production. Seed increase fields of the female lines are grown in an environment (e.g. short day and/or cooler temperatures) that represses expression of the male sterility genes, allowing the female lines to reproduce via self-pollination.

Cotton hybrids are widely grown in India, and also in China and Viet Nam), but very little elsewhere (Anonymous, 2005; James, 2002; Meredith, 1999; Roberts, 2005).

The Indian hybrids are produced by means of hand emasculation and pollination. The cost of labour for hand emasculation and pollination is prohibitively high in developed nations. Although genetic systems such as cytoplasmic-nuclear male sterility are present in cotton (Meredith, 1999), to date none of them have been used to make hybrid cotton on a large scale.

Soybeans (*Glycine max* (L.) Merr.) are not grown as hybrids, although nuclear and cytoplasmic-nuclear sterility systems have been reported as operational in the crop (Ding *et al*., 2002; Smith, Horner and Palmer, 2001). Effective cross-pollination systems must be developed (probably using insects such as honeybees (*Apis mellifera* L.) or leaf cutter bees (*Megachile rotundata* F.) and modified soybean flower morphology), to enable adequate levels of seed yield (Palmer *et al.,* 2001).

In theory one could produce apomictic hybrids of important field crops such as rice or maize; seed multiplication would be much less expensive and hybrids could be sold at lower prices. Additionally, farmers could reproduce the apomictic hybrids via saved seed as they now can do with pure-line self-pollinated crops. Research is underway to develop apomictic hybrids but the genetics are complicated.

Development of apomictic hybrids will require extensive research and experimen-

> *The components of apomixis comprise the absence of meiosis (apomeiosis), embryogenesis in absence of fertilization (parthenogenesis) and functional endosperm development … Reports on the genetic control of apomixis are often contradictory and show no clear consensus on the number of genes involved in the phenomenon. … The fact that most apomicts studied to date have suppressed recombination suggests that apomixis or apomeiosis is controlled by a tightly linked gene complex.*
>
> (Perotti *et al.*, 2004)

tation (Bi *et al.,* 2003; Hanna, 1995; Perotti *et al.,* 2004).

In summary, several of the major field crops in developed countries are grown extensively and often exclusively as hybrids. For example, maize, sunflower and grain sorghum are essentially 100 percent hybrid, and the area planted to hybrid rice and hybrid canola is growing rapidly. These same crops increasingly are grown as hybrids in a number of developing countries.

11.3 ADVANTAGES OF HYBRIDS

11.3.1 Increased yield and profitability for grower

For most crops, the best hybrids outyield the best non-hybrid cultivars, when both types are adapted to local conditions (Coors and Pandey, 1999; Pixley and Bänziger, 2004). Hybrids of self-pollinated crops benefit from an added component of yield: heterosis (hybrid vigour), unless the parents are very similar genetically. Hybrids of open-pollinated crops possess in reproducible form the higher yielding genotypes from among the wide range of hybrid genotypes present in any open-pollinated cultivar or improved population, and therefore the best hybrid will outperform the best OPC.

As noted above, farmers plant hybrids when the added income from higher yield of the hybrid more than compensates for the cost of the new seed required for each planting. The added income may be in-kind (as when the product is used directly as food or as animal feed) or in cash (when the product is sold on the market).

A caveat: the hybrids must be well adapted to the farmer's location and they must have preferred requirements in quality or other traits.

A second caveat: farmers will require that seed supplies (and suppliers) be reliable; seed deliveries must be adequate and timely, and seed must be of stated quality. In addition, commercial markets for the farmer's crop must be reliable and with adequate prices. If any of these criteria are not met, farmers will not use hybrid seed.

11.3.2 Increased dependability of performance

Successful hybrids are more tolerant of abiotic stress than the OPCs that they replace (e.g. Axtell *et al.,* 1999; Haussmann *et al.,* 2000; Menkir and Akintude, 2001; Pixley and Bänziger, 2004). They out yield the OPCs in stressful as well as high-yield environments in the location for which they are bred. In part this advantage is because heterosis *per se* can increase stress tolerance (e.g. Duvick, 2005; Mojayad and Planchon, 1994). But increased stress tolerance is also the inevitable product of breeding and selection for dependability of performance in the location where the hybrids are to be grown (Ejeta, 1988; Tollenaar and Wu, 1999). The most dependable hybrids, by definition, outyield other cultivars in low-yield as well as high-yield years, in unfavourable as well as in favourable growing sites

(Duvick, 1997). One should note that augmented stress tolerance as a result of breeding for dependability is not unique to hybrid breeding programmes. Breeding for dependable performance improves abiotic stress tolerance of non-hybrid as well as of hybrid cultivars (Rajaram, Singh and Ginkel, 1997; Smale *et al*., 2002), although heterosis *per se* plus ability to combine tolerance traits from two parents into one hybrid does give a unique advantage to hybrids.

A caveat: Breeding for tolerance of abiotic stress at one location does not necessarily give adequate tolerance to that stress in a markedly different location (e.g. the different locations may have a much greater intensity of the targeted abiotic stress, such as drought at flowering time).

11.3.3 Uniform expression of desired traits

Uniform expression of certain traits is highly desirable for some crops. Such uniformity is automatic in the case of cultivars of self-pollinated crops such as tomato or wheat, but not so for cross-pollinated crops such as maize or beets. Hybrids of cross-pollinated crops are uniform for all traits if the hybrids are single-cross hybrids, made by crossing two inbred lines. Thus, hybrids in cross-pollinated crops present new opportunities for producing a uniform product, if uniformity is desired or required, as in many horticultural crops (Wehner, 1999).

11.3.4 Greater range of useful traits

Where important traits (such as disease resistance) are controlled by dominant genes, a hybrid can contain (and express) the dominant genes from both of its parents, when neither of them has the full set of needed dominant genes (Wehner, 1999). The hybrid therefore will have better protection than either of the parents (more tolerance to biotic stress), and the goal of broad protection is reached in one generation rather than the long-term back-crossing and selection needed to place all of the needed genes in a single, inbred cultivar.

11.3.5 Customers can dictate traits of hybrid cultivars

Farmers who plant hybrid seed can dictate the traits of the hybrids they plant, if they buy the seed from commercial firms. Because the existence of commercial seed companies depends on seed sales, they must develop hybrids (or produce seed of hybrids developed by the public sector) that the customer wants to buy. For example, the hybrids must be adapted to the environment and farming practices of each adaptation region where the seed is to be sold, and additionally the hybrids must have specific traits and quality as desired by farmers, their potential customers.

Farmers may favour (or insist upon) maize hybrids with resistance to prevalent leaf diseases in their region, sorghum hybrids with tolerance to iron deficiency in alkaline soils common to their region, or rice hybrids with flavour and texture that suits their taste (or the taste of the ultimate consumers of their product). In the United States of America, during the past 70 years, maize breeders continually have had to breed hybrids with ever increasing tolerance to the stresses imposed by higher plant densities,

> *Scale of farmers is not that important. It is the desirable traits which farmers consider as important. If they are satisfied with the traits of the product, they do not mind buying [hybrid rice] seeds every year.*
>
> (H. Miah, pers. comm., 2005)

as farmers have gradually increased the plant density of their maize fields, from ca. 30 000 plants per hectare in the 1930s to the current 80 000 plants per hectare or greater (Duvick, 2005). Companies that fail to develop or distribute locally adapted hybrids with desired traits may fail as business entities, especially if competitor companies succeed where they have failed. Thus, the customer is in charge; breeders must do their best to furnish what the farmer wants and needs. [This is a clear example that client-oriented breeding does not necessarily include farmer participation. Editors.]

A caveat: if seed companies choose to not breed hybrids for a particular crop or region, farmers cannot force them to do so; in this case, public-sector breeding is essential if the farmers are to have hybrids [or farmers may be trained to produce hybrids for themselves. Editors].

11.4 DISADVANTAGES OF HYBRIDS

11.4.1 Annual capital investment by grower (seed purchaser)

Seed of hybrid cultivars must be purchased for each planting. As noted above, cash or credit must be available to farmers who wish to plant hybrids, and if that is lacking the farmer cannot grow hybrids. A corollary to shortage of cash is lack of a dependable and adequately priced market for the product. If the market is unstable, or offers prices that are too low, farmers will not make annual investments in hybrid seed. Such non-investment applies equally to other inputs requiring outlays of scarce cash or credit.

A second consideration is related to the kind of environment in which the crop is to be grown. Farmers may choose to not spend money on hybrid seed for planting in a potentially very unfavourable season (e.g. in the dry season in a region with alternating well-watered and very low rainfall seasons).

> *During winter season when … maize productivity is high, farmers in Bihar and eastern Uttar Pradesh in India, and in Bangladesh, are ready to buy hybrid seed, even though it is expensive. The same farmer will not plant hybrids in the same field during the summer season when risks of drought and floods are high and the chances of losing the crop are higher.*
>
> (G. Srinivasan, pers. comm., 2005)

Even though they plant hybrids in the favourable season, they will not do so in the unfavourable season; e.g. maize plantings in wet versus dry seasons in Lampung, Indonesia (Swastika *et al.*, 2004). They plant saved OPC seed in the risky (drought and/or floods) season because they know from experience that the crop may be totally lost or at best the yields may be so low that the cost of hybrid seed will not be matched by the value of extra yield of the hybrids. Similar considerations hold for farmers who grow their crop in other kinds of low-yield, risky environments (Pixley and Bänziger, 2004).

One might wonder why these farmers would not plant drought-tolerant hybrids instead of OPCs (presumably less tolerant to drought) in the unfavourable, dry season. Quite simply, the astute farmers know that their 'dry' seasons can be so disastrously dry that no amount of drought tolerance would suffice. They know that tolerance is not synonymous with immunity. The investment in hybrid seed would thus be totally lost. And even if the crop made some grain, the extra yield from the hybrids probably would not be enough to pay for the cost of the hybrid seed, as mentioned above.

11.4.2 Potential uniform susceptibility to new pest genotypes

As stated earlier, most hybrid genotypes are highly uniform. This means that they may be uniformly resistant to important diseases or insect pests, but they also may be uniformly susceptible to new and unexpected pests, or to an unexpected and highly unfavourable growing season.

This condition is not unique to hybrid cultivars; it is present and presents the same potential problems in self-pollinated crops such as wheat or soybeans (Simmonds, 1993; van der Plank, 1963). And even genetically heterogeneous crops—or wild plant species—can be devastated by a new genotype of insect or disease if they happen to lack the needed resistance or tolerance genes, despite their highly heterogeneous nature. Thus, Dutch elm disease (*Ophiostoma ulmi* Buisman) devastated the genetically diverse populations of native American elm (*Ulmus americana* L.) in the United States of America in the1960s (French *et al*., 1980). Chestnut blight (*Cryphonectria parasitica* (Murrill) Barr) overwhelmed the entire wild population of American chestnut trees (*Castanea dentate* (Marsh.)) in the mid-twentieth century (Anagnostakis, 2005). Ergot (*Claviceps purpurea* (Fr.) Tul.) infestations of rye (a genetically heterogeneous cross-pollinated crop) caused numerous epidemics of poisoning in Europe during the Middle Ages and in more recent centuries as well (Matossian, 1989). And many centuries before those events, the early Romans annually sacrificed a red dog to the god Robigus in hopes that he, sufficiently satisfied with the red dog, would not ravage their fields of genetically heterogeneous wheat cultivars with epidemics of red rust (*Puccinia* spp.) (see Large, 1982 for this and other narratives of destructive plant disease epidemics that resulted in widespread famine and great hardships for millions in the past). Genetic diversity can help, but it is not a cure-all.

Within any one season, farmers who plant uniform hybrids can spread their risk by growing several hybrids of differing parentage, more or less as when one grows a genetically heterogeneous non-hybrid cultivar. In addition, breeders constantly develop new hybrids of genotypes different from the predecessor hybrids, and thereby provide farmers with 'genetic diversity in time', also called temporal genetic diversity (Duvick, 1984; Smale *et al*., 2002). Also, as noted above, hybrids in self-pollinated crops can contain a broader range of resistance genes (greater internal genetic diversity) than is easily bred into individual pure-line cultivars.

Ultimately, however, one must recognize that to grow hybrids of cross-pollinated crops is to reduce the genetic diversity in the field, in comparison with growing highly heterogeneous OPCs of those same crops.

11.4.3 Difficult to serve unique small adaptation areas

Seed companies usually cannot afford to breed and produce seed for very small, specialized markets. Farmers may therefore be unable to find commercial hybrids that suit their needs if they are in a small region with

> *The challenge in developing countries is that [some farmers] have no or very limited choice because seed of improved [sorghum] varieties or hybrids is not available. Clearly, there are local preferences but it may be impossible for large companies to adequately address all of them.*
>
> (K. Porter, pers. comm., 2005)

unusual growing conditions or special quality requirements (unusual soil type, special cooking quality, etc.) (Paudyal *et al*., 2001).

One should note that professional breeding of crops of any kind—public sector or private sector, hybrid or non-hybrid—is likely to by-pass farmers in small, exceptional adaptation areas. The problem is not unique to hybrids; rather, the problem is that funds for breeding are always in short supply compared to the needs globally. Breeders, both public sector and private sector, ordinarily choose to breed for regions that hold the largest potential to use their products. As (or if) public funding or seed company sales prospects increase, the professional breeders gradually can afford to serve smaller and smaller adaptation areas. An advantage of public breeding organizations is that they intentionally can allocate scarce resources to breeding hybrids for farmers not likely to be served by the private-sector breeders (Bänziger *et al.*, 2004; Hassan, Mekuria and Mwangi, 2001).

11.5 BREEDING METHODOLOGY

11.5.1 Assembling and enriching breeding populations

Breeders of hybrid crops assemble and enrich breeding populations in ways that are not substantially different from those used by breeders of non-hybrid crops. When professional breeding gets underway in a new area, breeders usually use the best locally-adapted landraces for initial breeding and selection. Breeders of hybrids may develop a first generation of inbred lines from the selected landraces and, as well, form new populations (often enriched with elite germplasm from other locations) to be subjected to continual selection and improvement. They then use the improved populations or crosses among the first-generation inbreds

> *At CIMMYT we have [maize] gene pools and advanced populations which are derived from gene pools. Gene pools are broad based, formed from germplasm accessions, local varieties and diverse sources. We work with mild selection in gene pools [and] stringent selection pressure in population improvement (recurrent selection). We derive lines from [improved populations] and advance them as inbreds for further testing. At the same time, we also [practise] pedigree breeding in a limited scale.*
>
> (G. Srinivasan, pers. comm., 2005)

for further breeding and selection, and the cycle continues. Breeders at CIMMYT have used such methods to select drought tolerant maize for the tropics and sub-tropics; these improved populations then have been the source of inbred lines that can be the parents of superior hybrids (Bänziger, Edmeades and Lafitte, 1999).

It is not unusual for breeders of horticultural crops to make very wide crosses, such as interspecific crosses, to bring in novel traits (Rick and Chetelat, 1995; Tanksley *et al.*, 1996). This occurs with field crops as well: sunflower, maize and barley, for example, are crossed with wild relatives to bring in useful genes (Arias and Rieseberg, 1995; Grando, von Bothmer and Ceccarelli, 2001; Pons, 2003; Seiler, 1992; Whitt *et al*., 2002). Again, this practice is not unique to hybrid breeding but as noted above, introgressed genes may be used more easily in hybrids.

11.5.2 Inbred line development

Breeders produce uniform inbred lines to use as parents of hybrid cultivars by performing self-pollination in improved populations or in crosses of elite inbred lines (usually, lines that were parents of

Most of [maize] breeding populations are from pedigree breeding, some from backcrossing, little with improved populations.
(T. Kunta, pers. comm., 2005)

To a large extent we use pedigree breeding [for tomato and pepper], using elite hybrids. … We will occasionally use backcrossing if we want to introgress a new trait or disease resistance.
(R. Heisey, pers. comm., 2005)

In canola, most breeding programmes use doubled haploidy as a method of inbred development. However, pedigree breeding is still used in canola. Two-way, three-way and complex crosses are produced to generate inbred lines. Utilization of genetically broad populations or synthetics to extract inbred lines is not that common in canola. Backcrossing is mostly used in trait transfer.
(J. Patel, pers. comm., 2005)

successful hybrids). The latter method is called pedigree breeding, and for most crops it is the most widely used method for inbred development because it has higher odds of producing improved new inbred lines. At the same time, superior inbreds from improved populations (even if few in number) can bring in radically new and useful genotypes and form the basis for new advances in pedigree breeding or population improvement; thus, both methods are needed for continuing forward progress in a breeding programme.

Inbreds are selected for desired phenotypic traits during selfing generations. In field crop breeding they also are evaluated in test crosses (crosses to proven inbred lines) in order to select those with the best combining ability for yield and other important traits. The best lines from those small-plot trials are then crossed to other superior inbred lines to produce experimental hybrids that will themselves undergo several rounds of testing and elimination. Finally, a favoured few of the experimental hybrids will be chosen for introduction as new commercial hybrids.

At present, commercial hybrids of most crops are single-cross hybrids, i.e. a cross of two inbred lines. However, the first maize hybrids in the United States of America (1930s) usually were double-cross hybrids (cross of two single-crosses) or occasionally three-way crosses (single-cross female × inbred male). The double-cross method was used because the earliest inbred lines had very low and unpredictable yields and as a consequence the expense of producing single-cross seed was prohibitively high. Using a single-cross as a seed parent allowed production of hybrid seed at prices farmers could afford (Jones, 1918; Jones, 1922). Subsequent generations of inbred lines had incremental increases in yield (e.g. Duvick *et al*., 2004); eventually, inbred yields were high enough to allow the use of inbreds as seed parents for production of single-cross hybrids, hybrids that could be produced and sold at prices farmers could afford. Interestingly, a similar pattern has been followed with hybrid carrot (*Daucus carota* L.); later-generation inbred lines are more vigorous than the earliest generations, and

Since canola is an oilseed crop with quite a few important quality traits … in breeding nurseries we select for … high oil, high protein, low glucosinolates and low total saturated fatty acids.
(J. Patel, pers. comm., 2005)

thereby enable production of single-cross instead of three-way hybrids (Simon, 2000).

An exception to the progression toward single-cross hybrids can exist in regions with extremely low yield potential for field crops such as maize. In such situations, the lower yield potential of a double-cross hybrid (compared to the best single-crosses) may be acceptable if the lower seed price of the double-cross more than compensates for its lower yield (Hassan, Mekuria and Mwangi, 2001).

11.5.3 Assignment of inbreds to parental groups

As hybrid breeding programmes mature, breeders tend to sort inbreds into two or more groups based on their complementary interactions (Melchinger and Gumber, 1998).

Maize breeders called these groups 'heterotic groups', with the assumption that maximum heterosis for yield is achieved when inbreds from complementary groups are hybridized. At the least, inbreds in one group have a minimal genetic relationship with those in other groups, and so inbreeding depression is avoided when lines from one group are crossed with those of another. Perhaps a more provable reason for formation of the contrasting groups is that inbreds from one group can supply strength in traits that are not expressed or weakly expressed in inbreds of the other group (Tracy and Chandler, 2004). Thus one group might contribute better seed quality traits (and make good females), the other group might be better at pollen shed (and make good males). Or one group might contribute dominant resistance to certain disease or insect problems and the other group might contribute dominant resistance to a different set of important disease or insect problems. The hybrids would express the dominant traits of both sides and therefore would have a range of resistance greater than any of the inbred lines.

> *We take [sorghum] lines through a year of topcross, then move [them] to more wide area testing in more hybrid combinations. That will continue for 2–3 years and then we move to larger-scale testing in farmers' fields (strip trials) before release. Most companies do the same. Hybrids are screened in various screening trials for disease and insect resistance, lodging, drought screens, etc. Yield measurement is done at all stages of testing.*
>
> (K. Porter, pers. comm., 2005)

> *[We] use heterotic [single cross] testers ($A_1 \times A_2$ and $B_1 \times B_2$) which in the wider sense match the Tuxpeño/ETO pattern and have been chosen due to their excellent general combining ability, in addition to their excellent specific combining ability with each other.*
>
> (M. Bänziger, pers. comm., 2005)

Similar situations can exist in other crops, both field and horticultural. In some horticultural crops such an increased range of useful traits is the chief reason for making the hybrids; a hybrid may show very little heterosis for yield *per se* but it will have a greater range of desirable traits than is found in either of its non-hybrid parents.

11.5.4 Hybrid formation, trials and evaluation

Experimental hybrids are tested in various ways before some of them are chosen for production and sale to farmers. Breeders of field crops typically will test large numbers

Since there is minimal heterosis … in tomatoes and peppers … we do not try to make up heterotic groups. … We do however look for combinations of parental lines that exhibit "specific combining ability" for what I refer to as "economic heterosis". … An example of [economic heterosis] in tomatoes is the trait of "heat set" for the Florida growers, where a parent line with small fruit but which sets well under high temperatures is crossed with a line with very large fruit which sets poorly under high temperatures. The hybrid would have fruit of acceptable size, and moderate levels of set under high temperatures.

(R. Heisey, pers. comm., 2005)

Very extensive yield trialling [of maize hybrids] across the [Asia-Pacific] region; … minimum of 4–5 years and across representative areas/environments (30–50 locations per year at pre-commercial stage) … before releasing."

(T. Kunta, pers. comm., 2005)

of experimental hybrids in two or more seasons of small-plot yield trials, discarding all those that do not meet predetermined levels of excellence for yield and other necessary traits such as tolerance to locally important abiotic and biotic stresses. Trials are grown not only at the breeder's research station but also on farm fields distributed about the locations where the hybrids will be grown commercially. This ensures that trials are grown with management typical of that used by local farmers, as well as giving opportunity for the breeder to monitor performance of the experimental hybrids in the local environment.

Some of the trials may be conducted in "managed stress environments" to enable greater intensity of selection for tolerance to critical stresses (e.g. drought) of the target region (Bänziger *et al.*, 2004; Edmeades, Bänziger and Cortes, 1997).

During the performance trials, hybrids are rated for quality traits that are important for the crop, such as flavour and texture in rice, or oil quality in canola. After two or three seasons of small-plot yield trials, the surviving hybrids, now becoming potential commercial hybrids, are field-produced in limited quantity and distributed to farmers for evaluation by farmers (and breeders) at field scale under farmer-managed conditions. Finally, those few hybrids that have shown superior performance for all needed traits in both small-plot and farmer trials will be released and offered for sale. An essential element of "superior" performance is "reliable" performance; the hybrids must outperform other cultivars in both good and bad growing conditions of the adaptation area in which they are to be sold.

Such detailed testing may not be performed when hybrids are first offered for sale in a new region or country. Hybrids that are adapted to similar environments elsewhere will be introduced in limited amounts and those that do best will be sold in larger quantity (should there be demand for them). As (or if) the market grows, local breeding and testing programmes may be instituted to increase the numbers and adaptation of hybrids for the region.

Breeders of horticultural crops follow trial regimes similar to those for field crops except that they may move promptly from initial trials on their own research facility to distribution of limited amounts of experimental hybrids to producers, sufficient for the farmers to grow and evaluate the hybrids on a field scale. The farmers can compare the experimental hybrids with

their current favourite cultivars and report their findings to the breeder (R. Heisey, pers. comm., 2005).

11.6 SEED PRODUCTION

11.6.1 Technical aspects

Seed production techniques, such as detasselling, cytoplasmic male sterility, hand emasculation and pollination, and various kinds of self-incompatibility, have been discussed earlier.

All of the methods that use pollination by wind or insects on a field scale share a common need: to establish sufficient isolation from other sources of pollen to ensure that out-crossing is held to acceptably low levels. For some crops this can mean that seed production fields must be long distances from other fields of that crop.

Seed producers also must ensure that seed has satisfactory germination, is produced in sufficient quantity to satisfy farmer needs, and, importantly, is delivered in timely fashion, suited to the planting schedules of the farmers who use the seed. Truthful labelling is essential as well; this may seem to be an unnecessary statement, but in some parts of the world seed has been sold as first-generation hybrid when it was not. This seriously damages farmers' impressions of the value of hybrid seed and the veracity of seed companies (e.g. Ilagan, 2004) [and is particularly frequent in developing countries where Governmental Seed Companies have the monopoly of seed production and distribution. Editors].

Efficient and accurate performance of these multiple tasks requires skilled workers, specialized equipment, and, above all, a well-coordinated and well-directed organization, i.e. a hybrid-seed production company.

> *Testing [in sunflower] is necessary in all the zones of the world, mainly because each zone has some particular abiotic or biotic stress that needs to be assessed. Thus it is done in North America, throughout the sunflower growing regions of Europe, South America, India, Australia and other parts of Asia. Selection time from inbred genesis to culminating hybrid products can take a total of up to 10 years.*
>
> (G. Cole, pers. comm., 2005)

11.6.2 Seed producers

One should note that although public-sector breeders (e.g. academic institutions, government agencies or international research centres) do hybrid breeding (i.e. they may produce improved populations, inbred lines and well-tested final hybrids), the large-scale production and distribution of hybrid seed to farmers is nearly always the work of commercial seed companies.

Public-sector breeders (via the institutions that employ them) will release their plant breeding products for use by the private sector, using a variety of forms of release. In some cases, no restrictions are placed upon the released germplasm; in other cases, some sort of intellectual property protection is used to allow collection of royalties or other forms of payment (e.g. Butler and Marion, 1985; ERS, 2004; Heisey, Rubenstein and King, 2005; University System of Maryland, 2004).

Seed companies come in various sizes, from small local companies to large interna-

> *Because [sunflower] is a bee-pollinated crop, sizable isolation distances are required between varieties. Typically 1–1.5 miles [1.8–2.7 km] is utilized.*
>
> (G. Cole, pers. comm., 2005)

tional companies (Duvick, 2004; Fernandez-Cornejo, 2004). The large companies often are units of large international agribusiness corporations that deal in many products other than hybrid seeds. The small companies often supply hybrids to niche markets that are not easily served by the large companies, but they also may compete directly with large companies in large-scale markets such as hybrid maize in the United States of America.

A general pattern in the past has been for small companies to pioneer in development and delivery of hybrids for a given crop, usually with substantial help from public-sector breeders. Then, as the market matures, some of the small companies become very large and self-sufficient. Large agribusiness companies that may want to expand and diversify will purchase other seed companies. At the same time, numerous small seed companies also persist, successfully serving a local clientele.

To some extent this pattern is being followed today in some developing countries (López-Pereira and Morris, 1990). Small local seed companies are formed, or may expand their product line, to produce and sell hybrids adapted to their location; they often depend on public-sector breeders for germplasm in the form of inbred lines and recommended hybrid combinations.

However, the large international seed companies are usually present at the beginning as well (e.g. Hassan, Mekuria and Mwangi, 2001; Rusike, Howard and Maredia, 1997), and sometimes parastastal agencies perform the function of a commercial seed company.

Farmers in the state of Bihar, India once showed me some fields of obviously F_2 hybrid maize. They had purchased 'hybrid seed' from an itinerant salesman. They assured me that he had better not come around again.

(D. Duvick, pers. comm., 2005)

[Hybrid rice] seeds [in Bangladesh] are distributed through sales representatives of seed companies.

(H. Miah, pers. comm., 2005)

Evolution of the hybrid seed business in developing countries today does not exactly parallel that of the industrialized countries in earlier decades (e.g. Maredia and Howard, 1998).

11.7 SEED SALES AND DISTRIBUTION

Commercial seed companies employ a variety of ways to sell and distribute their product. Often, a company's local sales representatives will contact the farmers, counsel them on the merits and management needs of the hybrids they hope to sell, and arrange for the sale and delivery of product. In other cases, distributors and retailers of farm products provide similar services, but with more centralized sourcing and less personal contact with the farmer. The smaller and more isolated markets are more likely to be served by distributors/retailers.

11.8 UTILIZATION

11.8.1 Commercial crop production

Commercial producers of both horticultural and field crops will readily plant hybrid cultivars if they outperform the best non-hybrid cultivars, have desired quality traits, and are adapted to the local environment and to the management practices preferred by the producers. The usual caveat applies here, that the breeding of hybrids for farmers' needs must be consistent and

CIMMYT [maize] inbreds are used both by commercial (big) seed firms as well as by smaller seed companies in developing countries. The major difference will be that the big multi-nationals … use one of our inbred lines (as such or after further inbreeding and selection) in their hybrids along with a proprietary inbred from their programme, whereas many of the smaller seed firms have directly released [hybrid] combinations [of inbreds] developed and tested by CIMMYT.

(G. Srinivasan, pers. comm., 2005)

In Sudan today, annual acreage of hybrid sorghums has reached one million acres. This successful story of seed production was made possible primarily by a local parastatal, the Sudan Gezira Board.

(G. Ejeta, pers. comm. 2005

long term; seed supplies must be ample, of good quality and delivered on time; and markets and yield prospects must be sufficiently high that farmers can be assured of ample return on their seasonal investment in hybrid seed.

11.8.2 Home use crop production

In many areas of developing nations, both horticultural and field crops are grown by small-scale farmers for home consumption and may supply most or all of the family's food supply. Often crops are grown for two purposes: to furnish food for the home and also for sale on the market in order to bring in much-needed cash. An additional factor is that small-scale farmers in developing countries often reside in locations with relatively poor agricultural potential, such as on steep mountain slopes or droughty soils, or in unreliable climates. Yield prospects may be low and uncertain. In addition to these problems, these farmers are more likely to be offered relatively low prices for their produce because they are far from the ultimate consumer or have poor transportation to connect them to sources of consumption (e.g. Ekasingh *et al.*, 2004; Ha *et al.*, 2004).

For any of the above reasons, small-scale farmers might conclude that purchase of hybrid seed is a risky investment, a chance they cannot afford to take (Pixley and Bänziger, 2004).

Despite such problems and concerns, small-scale farmers who grow most of their own food have moved strongly to use of hybrid crops in many countries. Size

There is no hard and fast rule on consumption and selling of hybrid paddy [in Bangladesh]. Medium [size] farmers prefer consumption if they do not have pressure for selling … for immediate cash money. Big farmers sell most of their products. Commercial production of hybrid rice has no link with farm size. It is the choice of the farmers and the availability of seeds in time.

(H. Miah, pers. comm., 2005)

Some very small farmers buy our [hybrid pepper and tomato] seed, but only if they are able to sell some of the produce to offset the cost of the seed.

(R. Heisey, pers. comm., 2005)

[E]vidence began to accumulate that, despite the conventional wisdom, hybrids in some cases represent an appropriate technology even for small-scale, resource-constrained farmers.

(López-Pereira and Morris, 1994)

Sorghum hybrids are planted by farmers of all sizes. If farmers can recognize value, they will purchase the input … Farm size has little to do with it. Our experience is that farmers will find a way to purchase hybrid seed if it adds value to their operation … They may go together with a neighbour to buy seed and split it or they will make some arrangement for purchasing on credit from the local retailer.

(K. Porter, pers. comm., 2005)

of farm seems to make no difference, as long as the farmers have reasonably good assurance of favourable growing conditions for the crop and dependable markets if they want to sell some or all of their produce.

Of course small-scale farmers must have some source of cash income in order to pay for the seed, unless they buy it on credit with promise to recompense the seller with some portion of the resulting crop.

But despite a general movement toward use of hybrids for some crops, one must recognize that civil unrest and consequent social and fiscal constraints, or simply lack of well-adapted hybrids, can dictate against purchase and use of hybrid seed. In circumstances such as these, saved seed of non-hybrid cultivars may be the only prudent option.

11.9. PARTICIPATORY PLANT BREEDING FOR HYBRIDS

Breeding systems and the seed production and delivery systems for hybrids are often complicated and require large amounts of labour, capital and genetic expertise.

Farmers cannot easily produce all of these inputs by themselves. And even if farmers could perform all of the steps of hybrid breeding and seed production for themself, the annual cost in terms of land, labour and capital would usually be greater than if the farmers were to simply purchase their hybrid seed for each season's plantings (see Morris and Bellon (2004) for discussion of the trade-offs with participatory plant breeding).

Nevertheless, in certain situations, farmer participation in the hybrid breeding process may be essential or profitable, or both. For example, in some cases neither public nor private plant breeding organizations will have developed hybrids for a particular environmental niche or quality requirement (as noted earlier). In such situations, intensive farmer participation in selection of breeding materials as well as in evaluation trials may help to ensure selection of hybrids that fit the farmers' unique growing conditions, cultural methods or quality preferences. In other cases, local farmers or cooperative organizations, collaborating with public-sector (or private-sector) breeding organizations, may be able to produce and deliver appropriate hybrid seed to an under-served area.

An example with hybrid wheat: "Conversion to a sterile cytoplasm and the development of restorers and maintainers would take much time and skill. Use of a chemical hybridizing agent would require access to the chemical, spraying equipment, and also add challenges of timing and efficacy with different genetic backgrounds. Furthermore, the need for large isolated crossing blocks and alternating strips of pollen parent necessary for wheat would use land and resources in small farming communities that might not be able to afford that use of productive land."

G. Marshall, pers. comm. (2005).

It will be instructive to examine the possibilities for participatory plant breeding at each of the several stages of hybrid breeding and seed production and delivery as discussed earlier. The following comments briefly discuss possibilities for each stage.

Assembling and enriching breeding populations

Professional breeders in public and private breeding programmes are best suited to survey the global supply of useful breeding materials and to assemble appropriate materials for further breeding. However, farmers in developing countries may be best suited to identify and contribute germplasm uniquely adapted to their own environmental or quality requirements, especially if they farm in niche environments or have unique quality needs (see Sperling *et al*., 2001). Professional breeders could use the farmer contributions as key ingredients of new breeding populations intended for use in developing hybrids for those farmers. This method, in a simplified version, was the basis for breeding of hybrids in the industrialized countries when hybrid breeding began in the early years of the twentieth century. Inbred lines were selfed from highly regarded OPCs (e.g. maize breeders in the United States of America selfed favoured farmer varieties such as Reid, Krug and Lancaster); the inbreds were combined into hybrid combinations; and, following evaluation trials, the breeders selected the best hybrids for production and distribution (see Jenkins, 1978).

A caution: in today's environment of intellectual property rights and farmers' rights, proper attention will be needed to ensure that local laws and regulations are obeyed in the process of contribution and use of farmer OPCs (see discussion in Morris and Bellon, 2004).

Inbred line development

Professional breeders are best suited to grow and self-pollinate hundreds or thousands of rows of inbred lines at various stages of inbreeding, rate them for desirable traits, and eventually discard all but a handful of the best new lines. But farmers, if they could spare the land and labour, could provide useful information in a collaborative fashion by growing duplicate rows of the same materials (partially selfed inbreds, *per se*) for observation of performance under their specialized conditions. Breeders could use this information as valuable supplemental information to assist their save or discard decisions in the course of inbreeding.

Farmers, especially poor small-scale farmers in developing countries, would probably not wish to do such collaboration, however, because the inbred lines would use space needed for regular crop production but usually would yield much less than OPCs or hybrids. The farmers' income would be reduced to unacceptable levels. Perhaps the farmers could be paid (in cash or in kind) to perform such a collaborative service.

Professional breeders might be able to justify such an outlay if it added to the speed and precision of their breeding effort. But they too might find the extra effort was not worth the expense except in cases where it was impossible (or too expensive) to reproduce unique abiotic or biotic stresses that occurred in the farm settings.

Also, as noted above, considerations of intellectual property rights might mitigate against such distribution and handling of potentially valuable proprietary germplasm.

Assignment of inbreds to parental groups

Professional breeders, once again, are best suited to perform this operation, using

their extensive knowledge of pedigrees, performance of inbred lines in various hybrid combinations, and, increasingly, the information in genomics databases.

Hybrid formation, trials, evaluation

Professional breeders can best organize and carry out these tasks, although farmers can (and should) participate in the evaluation stages. Annually, breeders make many new experimental hybrids, with full knowledge that only a few of them will perform well enough to save and release as acceptable new hybrids. Typically, one must make dozens or hundreds of experimental hybrids for initial observation and yield trials. As noted earlier, a few seasons of performance and evaluation trials in appropriate environments will enable the breeders to reduce the number of experimental hybrids to a much smaller total. This reduced number of hybrids ('advanced experimental hybrids') can then be tested much more widely, in particular in those environments and locations where they are to be grown and used by farmers.

Farmers and breeders can, and do, collaborate in conducting many of these performance trials, especially in the more advanced stages of selection. Such collaboration is common today in industrialized countries, as well as in some regions of developing countries. For example, from the first days of hybrid breeding, seed companies in the United States of America have grown the majority of their advanced small-plot hybrid yield trials on land of collaborating farmers. The professional breeders provide seed, plant the trials and harvest them; the farmers provide land and cultural practices. Sometimes the farmers have received some kind of payment, for example if the trials on average yielded less than the farmer's commercial crop. Breeders use the small-plot information to guide them in evaluating the adaptation of hybrids to particular environments, cultural practices and farmer preferences. Farmer participation is passive in the sense that farmers make no decisions about which hybrids are entered in the trials or which ones are saved, and therefore is one type of Participatory Variety Selection (PVS) (Chapter 3).

Another step in collaborative evaluation in the United States of America came about after farmers adopted combines for harvest, starting in about the 1970s. Experimental hybrids in the final stage of trials are now widely tested by farmers in 'strip trials'. Farmers plant field-length strips (e.g. several rows) of experimental hybrids (often furnished gratis by seed companies) next to strips of their favourite commercial hybrid(s), and at harvest time a measured length of each strip is combine harvested and the grain is weighed, tested for moisture, and yield is calculated. Other notes on pertinent traits may be taken at the same time.

Farmers use these strip-test data (typically those from their own farm and also those from neighbouring farms) to help decide which commercial hybrids to plant in their fields in the following season. Their participation is active in that they choose which hybrids to compare in strip trials and which hybrids to plant in the following season, but is still a type of PVS [Editors' note].

Seed companies use the strip-test data to help them decide which experimental hybrids to save and promote to commercial status (i.e. production and sale) for the following season.

Thousands of such trials are performed each season. Properly analysed, the data can be statistically significant, and can help the breeders to characterize the specific adaptation(s) of each hybrid. They are a

valuable addition to the data gathered from traditional small-plot yield trials (for more detail, see Duvick, 2002).

Such collaboration, for small-plot trials or for strip-test trials, can be more difficult to carry out in developing countries, with very different logistics, economies, and farming systems, especially for small-scale farmers. But with appropriate design, organization and supervision, private or public breeders can collaborate with farmers to carry out performance trials of advanced experimental hybrids (see Chapter 9). As in the industrialized countries, the later stages of hybrid selection (when numbers of experimental hybrids are reduced to dozens or fewer, rather than hundreds) will be appropriate for such collaborative trials.

CIMMYT maize breeders have instituted an effective collaborative system of this kind ('Mother-Baby' evaluation trials) in Zimbabwe (Bänziger and de Meyer, 2002). Without unduly taxing farmer land and labour, the system enables selection of hybrids best suited to the local weather, soils and farming methods, educates the farmers in possibilities for hybrid use and value (the trials include OPCs as well as hybrids), and also feeds farmer knowledge and preferences back to the professional breeders.

The two-way interaction, among other things, guides the breeders in selecting appropriate traits and trial conditions for the earlier stages of hybrid breeding and evaluation trials (operations that are performed entirely by the professional breeders). The Mother-Baby trials have produced interesting and useful by-products.

National agricultural research programmes in other southern African countries have adopted versions of the Mother-Baby trials.

> *The Mother Trial was a replicated researcher-managed trial, planted in the centre of a farming community, typically with a school or a progressive farmer. It evaluated 12 cultivars under two input levels, using two-row plots and three replications. Baby Trials were grown by at least six farmers in a community that hosted a Mother Trial. Each Baby Trial contained four of the varieties evaluated in the Mother Trial and all entries in the Mother Trial were represented among Baby Trials. Farmers were requested to treat the four cultivars uniformly but follow their own management practices. … Trial entries came from several public and private breeding programmes chosen by the breeders "as being the best bets for smallholder farmers' conditions". This [method was used] because of the project's goal of exposing farmers to new varieties.*
>
> (Bänziger and de Meyer, 2002)

> *Local farmers and partners suggested that information from Mother-Baby Trials should be made available to retailers to increase the availability of appropriate varieties.*
>
> (Bänziger and de Meyer, 2002).

A less formal but also effective method of participation (used in vegetable breeding) is described below.

Seed production

As noted in the earlier discussion of this topic, the sum total of the logistics, equipment and technical skills required for production and distribution of hybrid seed requires the establishment of formal organizations, and these, typically, have been commercial seed companies. The

> *We do a lot of inbred development and hybrid evaluation in developing countries such as Guatemala and Jordan by working on farms (usually small by our standards) owned and/or run by our dealers and cooperators. We end up with hybrids that are adapted not only for growing by the larger farmers, but also for the marginal farmers*
>
> (R. Heisey, pers. comm., 2005).

seed companies may produce hybrid seed made primarily from inbred lines that are developed by public-sector organizations or that are leased to them by private-sector firms. Alternatively, they may have their own extensive breeding establishment and produce (and primarily depend upon) their own proprietary inbreds and hybrids. Small companies typically follow the first path; larger firms usually take the second route.

There is a third possibility. Farmers can form cooperatives to produce hybrid seed for themselves; they can use inbreds from the public sector in approximately the same way as is done by the smaller commercial firms. Although cooperatives come in various forms, one can use the definition: 'a jointly owned commercial enterprise (usually organized by farmers or consumers) that produces and distributes goods and services and is run for the benefit of its owners.' Thus, although the members of the cooperative would control its activities and share in its benefits, the cooperative also would be a commercial company in the sense that it would market seed to farmers.

A drawback to formation of cooperatives for hybrid seed production and distribution is that substantial amounts of capital and skilled personnel would be required. Training in hybrid seed production might come from public-sector organizations (or appropriate NGOs) and loans of capital might be arranged through suitable government organizations. But these requirements in capital and training of personnel could be significant or even impossible obstacles for poor farmers in countries or regions with unstable government and economy.

A second important consideration is that the seed production cooperative would need continuing services, through the years, of a public-sector organization to develop inbreds and hybrid combinations. The public-sector establishment would need to provide a constant flow of new inbreds and hybrids to keep up with changing disease and insect problems, and perhaps changing abiotic challenges as well. This assumes that the cooperative would not do its own breeding, or contract with the private sector for such materials. Without such a source of inbreds and hybrids, there would be no justification for forming a cooperative.

An experienced sorghum breeder has given a concise summary of requirements for a hybrid seed production cooperative as follows:

Farmer-organized hybrid seed companies were formed in the United States of America in the early days of hybrid breeding. For example, Dr H.C.M. Case (professor at the University of Illinois) and five farmers from Champaign County, Illinois, organized the Champaign County Seed Company, in 1937 (Widick, 2005). The organization was formed to 'grow, condition and sell hybrid maize seed' as one of the 'associated growers' of Lester Pfister. Pfister was an entrepreneurial farmer-breeder of maize hybrids who had enlarged the seed production and sale capacity of his company (the Pfister Hybrid Corn Company) by forming Pfister Associated

Cooperatives can produce their own seed if they are willing to invest in the seed production elements that result in quality seed. These are: (1) a mechanism to ensure getting pure seed of inbred parents; (2) training of qualified seed plant managers; (3) identification of reliable farmer seed growers; (4) hiring people to manage the various aspects of seed production (planting dates to ensure isolation, removing rogues, proper harvest techniques, etc.); (5) acquiring facilities for drying and conditioning the seed for maximum quality; (6) evaluation of final quality (germination, vigour test, purity, etc.); (7) proper packaging; and (8) storage facilities.

(K. Porter, pers. comm., 2005)

Growers (P.A.G.), consisting of approximately two dozen independent seed production enterprises like the Champaign County Seed Company. P.A.G. eventually reorganized as an independent, relatively large-scale conventional seed company with its own breeding programme. In due course, an even larger company purchased P.A.G. (Fernandez-Cornejo, 2004).

Despite the potential difficulties discussed in previous paragraphs, farmer cooperatives have been formed in developing countries to produce hybrid seed.

The Philippine cooperatives appear to be transitioning into small seed companies that depend on public-sector breeders for inbreds and hybrids.

A different way for farmers to participate in (and profit from) hybrid seed production is to produce hybrid seed on their farms, on contract to commercial seed firms. In India, hundreds of farmers in Andhra Pradesh produce hybrid maize seed in this way, guided and trained by a local contractor, or by the seed companies directly. The seed production is said to be economical and of high quality. The farmers are compensated on the basis of the amount of seed they produce for the contracting company. This enterprise 'is a huge source of income and contribution to the local economy' (G. Srinivasan and D. Beck, pers. comm., 2005).

Farmers' cooperatives in Viet Nam have played a role in the hybrid rice seed industry for some time now. The seeds harvested are turned over to the provincial seed companies that in turn sell the seeds to the farmers. There are now nine community-based farmers' cooperatives for hybrid rice seed production in the Philippines. They mainly produce seed of the public-sector-developed hybrids. The national research institute provides technical backstopping whenever needed by the cooperatives. The Philippine Rice Research Institute, which is responsible for the development and dissemination of the technology, facilitated the establishment of these cooperatives. It was responsible for the development and dissemination of the technology. In the beginning, seeds produced by the cooperatives were procured by the government and sold to the farmers at subsidized prices. The subsidy is now gradually being phased out and the marketing of the hybrid seeds is transferred entirely to the cooperatives. The sustainability of this arrangement remains to be seen since seed marketing has been only recently transferred to the cooperatives

(R.S. Toledo, pers. comm., 2005)

Seed sales and distribution

As noted above, cooperatives might not only produce hybrid seed, they might distribute

it as well. To maximize the profitability of their investment in production facilities and trained personnel, they might choose to sell to a broader market than only the members. At this point, it would be hard to distinguish the cooperatives from traditional small seed companies.

General comments in regard to participatory plant breeding for hybrids

Farmers have fewer opportunities to collaborate with professional breeders in the several stages of the hybrid breeding, production and distribution process than is possible for breeding of non-hybrid cultivars. Possibilities for farmer participation are primarily in hybrid evaluation and, to some degree, in seed production and distribution (e.g. farmer cooperatives or contract seed production).

The use of cooperatives for seed production and distribution requires (i) formation of a well-managed corps of skilled operators; (ii) meticulous and sometimes difficult hybridization operations; and (iii) well organized and often expensive facilities and distribution systems. This complicated seed production and distribution step is not needed, or can be done more simply, in participatory breeding of non-hybrid crops, e.g. in production of farmer-saved seed. Seed production and distribution of hybrids is best performed by organized business entities.

Nevertheless, when all is said and done, the facts are that individual farmers can and do participate constructively in some of the stages of hybrid breeding, production and distribution. In so doing they help themselves—they help to ensure the availability of hybrids that suit their needs and financial status.

In the absence of a viable seed industry, the means to get hybrid [sorghum] seed produced and marketed will, I believe, have to come from within the farm community. [But] seed production is a vital knowledge and experience that needs to be built up.

(G. Ejeta, pers. comm., 2005)

Each crop is different. The farmer/breeders will need a great deal of assistance on many things … producing, harvesting, processing, and marketing the seed.

(W. Meredith, pers. comm., 2005)

[The] Bangladesh perspective, in limited scale, is to invite farmers in at several stages of plant growth to watch the hybrid fields managed by plant breeders. Farmers are at liberty to give their opinions … and breeders seriously consider them in their further course of action. … For seed production, one NGO (Rangpur Dinajpur Rural Service) organized women farmers who were trained in the Bangladesh Rice Research Institute. These groups of women farmers did a marvellous job in producing quality seeds.

(H. Miah, pers. comm., 2005)

We find that farmers (even small-acreage farmers) are very interested in helping with the research programmes by providing land for growing breeding nurseries or screening nurseries. They are anxious to assist with hybrid testing and evaluation and provide excellent input on specific traits that impact their profitability. They also provide excellent input and comments as we visit with them during crop tours and research trial visits.

(K. Porter, pers. comm., 2005)

11.10. CONCLUSIONS

Breeding and use of hybrid cultivars has increased worldwide and in significant amount during the past several decades, in part because of continuing development of effective and economic systems for hybrid seed production and in part because of continuing genetic improvements in performance and profitability of the hybrids (e.g. Axtell *et al.*, 1999; Duvick, 2005; López-Pereira and Morris, 1990). Hybrids work for many crops, but not all; they work in many farming regions of the world but not all farming regions; and they work for many farmers, but not all farmers.

It seems likely that in the years to come, hybrid development, distribution and utilization in all parts of the world will depend primarily on effective services of a diverse array of private seed companies. Some companies will carry out the entire spectrum of breeding and evaluation, seed production, and delivery, while others, such as smaller companies and farmer cooperatives, will depend to a greater or lesser degree on inbred lines and improved germplasm from the public sector (or, at times, leased from other companies in the private sector). In addition to the ongoing need for public-sector breeding of inbreds and hybrids, there will be a continuing need for the fundamental products—both germplasm and knowledge—that are provided by able public-sector professional plant breeders. Public-sector contributions will be especially important for development and provision of hybrids to small-scale farmers in some of the disadvantaged farming regions of developing countries.

Farmers will participate in hybrid breeding. Their participatory plant breeding activities will be essential and beneficial to farmers and breeders alike, but farmer participation will be less than is feasible in breeding of non-hybrid cultivars.

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CHAPTER 12

Selection methods Part 4: Developing open-pollinated varieties using recurrent selection methods

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12.1 INTRODUCTION

This chapter provides an overview of the use of recurrent population improvement methods in variety development. The major stages of population improvement are addressed, from setting objectives and population creation, through progeny development and selection, to recombination. Factors contributing to successful use of recurrent population improvement methods for participatory variety development are provided, and examples given of farmers' contributions to these efforts.

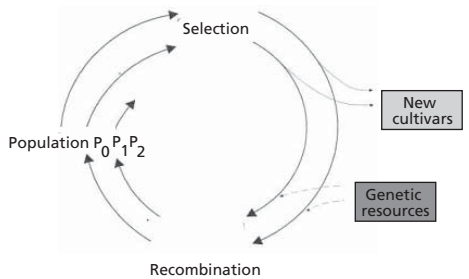
12.2 RECURRENT SELECTION: WHAT IS IT AND HOW DOES IT CONTRIBUTE TO VARIETY DEVELOPMENT?

Recurrent selection schemes involve cycles of testing, selection and recombination of breeding 'units', with the possibility of deriving new varieties from each population cycle bulk or from the progenies developed during each cycle (Figure 12.1). Repeated cycles of selection are conducted to increase the frequency of desirable alleles in a population, and obtain progenies that are superior to the best progenies of the previous cycle (Figure 12.2). Ideally, the genetic variability in the population is maintained and thus further genetic gains can be achieved in subsequent cycles.

Recurrent selection methods are readily applied in out-crossing species, where ease of crossing facilitates the frequent and extensive recombinations required in these schemes. The extensive crossing can be more laborious in self-pollinating crops, and requires committed efforts for extensive emasculated crossing or the employment of male-sterility genes or selection for higher out-crossing rates to facilitate recombination.

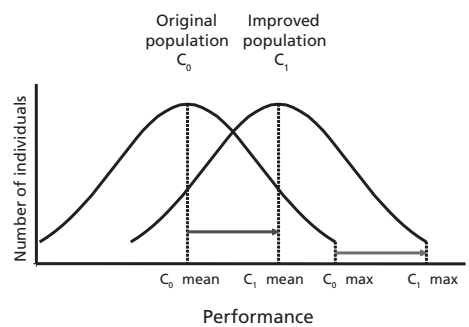
The basic scheme of recurrent selection is presented in Figure 12.3, with terminology proposed by Strahwald and Geiger (1988). The plants or progenies that are selected to constitute the next, hopefully improved, population bulk are termed selection units. Selection is based on the performance of individual plants or progenies, named evaluation units, for a single trait or an index of several traits. The next cycle of the population is created by inter-mating the selected plants or progenies which are called the recombination units. These different

FIGURE 12.1
Schematic presentation of an open recurrent selection scheme with cyclic selection and recombination combined with introgression of new germplasm



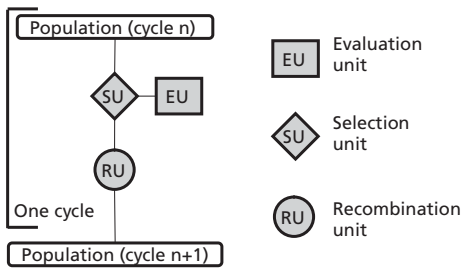
Becker, 1993.

FIGURE 12.2
Expected performance distribution of progenies from the original and an improved population



After Fehr, 1987.

FIGURE 12.3
Generalized population improvement scheme for advancing a population from one cycle to the next



From Strahwald and Geiger, 1988.

units are related to one another or may even be identical, depending on the selection method used. For example, in simple mass selection in a highly cross-pollinating species, the selected S_0 plants are the evaluation and selection units as well as the recombination units, with the half-sib seed of the selected plants used to produce the next cycle bulk. A more complicated example would be an S_2 population improvement scheme where S_2 progeny bulks are used as the evaluation units in order to have sufficient seed for multi-environment testing. The selection units and recombination units in this case could be the original S_1 progenies, when remnant S_1 seed of the superior S_2 bulks is used for recombination.

A wide range of recurrent selection methods are available, with alternative methods normally identified by the progeny type used as the test unit (Table 12.1), (Hallauer and Miranda, 1981; Gallais, 1981). The choice of selection scheme depends on the type of end-product desired (improved population or pure-line varieties) and the traits to be improved. It also depends on the crop species to be improved (autogamous or allogamous) and the resources and costs (e.g. labour, test site facilities) the breeder can apply for the recurrent selection. For example, the development of pure-line varieties with high grain yield performance could be more successfully pursued by S_1 or S_2 selection methods using multi-location testing of a very large number of test units that more closely resemble the desired end product. In contrast, the development of more genetically heterogeneous varieties of an allogamous species could well be

TABLE 12.1
Alternative recurrent selection methods, required number of generations (or years), and degree of exploitation of the variance of additive (σ^2_A) and dominance (σ^2_D) effects

Selection method	Generations per cycle	Genetic variance σ^2_G	
		σ^2_A	σ^2_D
Phenotypic (mass)[1] With recombination			
One sex (after flowering)	1	1/2	1
Both sexes (before flowering)	1	1	1
Half-sib			
Selfs recombined	2	1/2	0
Half-sibs recombined	2	1/4	0
Full-sib	2	1/2	1/4
S_1-line	3	1[1]	1/4[2]
S_2-line	4	3/2[1]	3/16[2]

Notes: [1] Not equal to σ^2_A unless p = q = 0.5 and dominance decreases to zero with inbreeding.
[2] Coefficient difficult to define unless p = q = 0.5.
Source: adapted from Schipprack, 1993.

done by applying mass selection for simply inherited traits.

The genetic diversity that is retained in the improved populations enables continued improvement. Several cycles of directional selection should increase the frequency of desirable alleles, resulting in higher probabilities of obtaining superior progenies than in the original population (Figure 12.2). Furthermore, the genetic variability retained in a broad-based population enables selection for different traits or adaptation to new target conditions as the needs emerge. Examples of this are listed below.

- A broad-based population retaining substantial diversity for maturity or other adaptive traits could be given to breeders (researchers or farmers) in differing agro-ecological zones, to develop zone-specific populations.
- A population retaining variability for grain quality traits could be used to derive distinct populations for other quality requirements.
- Rare dwarf segregants in a tall-plant-height population can be selected to develop a new short-plant-height population with potential for increased grain harvest index.

12.3 EXPECTED GENETIC GAIN OR THE RESPONSE TO SELECTION

The response to selection (R), using any type of selection, is a function of the intensity of selection (i), the extent to which observed differences are determined by genetic causes (h = square root of heritability) and the extent of additive genetic variation (σ_A), as indicated by the formula (Falconer, 1981):

$$R = i^* h^* \sigma_A$$

The different recurrent selection methods listed in Table 12.1 vary fundamentally for the degree to which they can exploit the available additive genetic variance (σ_A). The other two factors (selection intensity and heritability) can be managed by breeders to optimize genetic gains. Options for managing these factors will be discussed in more detail in the sections on mass- and progeny-based selection methods.

It is crucial to consider the time to complete a cycle of selection since the amount of progress is determined both by the gain per cycle and the time per cycle. Table 12.1 indicates the minimum number of generations required for various methods. The time required for one cycle can be reduced significantly if off-season nursery facilities are available.

12.4 BREEDING OPEN-POLLINATED VARIETIES USING RECURRENT SELECTION METHODS

Varieties of cross-pollinated species can be developed by selection in a population bulk *per se*. This approach is appropriate where intra-varietal heterogeneity is desirable or necessary. Varieties can be derived from the population bulk *per se* by mass selection for specific highly heritable traits that give the variety a more distinct character; for example, a narrower range of flowering dates or more uniform plant height, or grain or plant colour. The highly successful pearl millet variety ICMV 155 was created by this method, with 59 S_0 plants mass selected during the random mating of the New Elite Composite Cycle 4 bulk used to create a new variety (Singh *et al.*, 1994).

Varieties of cross-pollinated species can also be developed from a set of superior progenies identified during the selection phase of progeny-based recurrent selection. For each variety, a separate set of progenies would be identified based on a

distinct selection criterion or combination of traits, and the progenies in this set would be recombined to create the new variety. Several different varieties can be created in this manner from a given set of population progenies by selecting for different trait combinations or placing different emphasis (weighting) on the targeted traits. Examples of successful pearl millet varieties developed in this manner are ICTP 8203, created by random mating 5 superior S_2-lines identified by progeny testing a large number of lines derived from a Togolese landrace at Patancheru, India (Rai *et al.*, 1990), and WC-C75, created from 7 full-sib progenies selected out of the World Composite (Andrews, Gupta and Singh, 1985).

Pure-line varieties for predominantly self-pollinating crop species such as sorghum can be effectively derived from the superior partially inbred evaluation units (for example S_1 or S_2 lines) identified in a progeny-based recurrent selection programme. Breeders usually follow the same procedures as for deriving lines from biparental crosses (Chapter 11, this volume).

Use of recurrent selection methods in variety development programmes can be particularly advantageous for enhancing quantitative traits determined by many genes, or simultaneous enhancement of multiple traits. A large number of favourable alleles can be carried forward and concentrated with repeated recombination, breaking undesirable linkages, and selection for favourable recombinants. Allard (1999) notes that the assembly of favourable epistatic combinations of alleles of different loci by means of recurring cycles of selection and intercrossing the superior selections is the single most important genetic mechanism for evolution of adaptation.

12.5 SETTING GOALS AND DEVELOPING BASE POPULATIONS

The success of any plant breeding programme is usually measured by the extent of farmer adoption of the newly produced varieties. As the specific advantages of new varieties determine adoption, breeders must tailor their new varieties to meet priority needs and requirements of the end users. Priority setting for a recurrent selection programme requires good understanding of the environmental conditions under which the newly developed varieties should perform, as well as of the needs of the farmers or end users expected to benefit from the new varieties. Methods and tools for effectively identifying and defining the priority targets for participatory variety development are provided in Weltzien, vom Brocke and Rattunde (2005) and in Chapter 4.

By explicitly defining the goals and expectations of a given population, parents can be selected that best contribute to the creation of the new population/variety with the desired genetic variability. Key questions for choosing parents include:

- What are the target environment(s), zone and group of farmers for whom the population should be of use?
- What is the acceptable range for critical adaptive and quality traits, such as maturity, grain type, biotic challenge resistances, and adaptation to specific soil and water regimes?
- What is the priority trait or combination of traits that are a target for improvement?
- What is the appropriate balance between level of diversity and eliteness?

The balance between level of diversity and eliteness is a critical issue in the choice and number of parents used for developing the population or variety. Maximizing the

diversity of the population through selection of parents with outstanding performance for certain traits but less desirable for others will maximize the potential for long-term genetic gains, but reduce the possibility of deriving agronomically superior end products in the short term. In contrast, greater emphasis on population 'eliteness' through more restrictive inclusion of parents for population creation will maximize opportunities for immediate extraction of distinct finished varieties, but limit long-term potential gains and benefits from intra-varietal diversity.

12.6 MASS SELECTION

Mass selection involves the selection of individual plants or even of individual grains or seeds (Allard, 1999). This type of selection is based on the phenotype only, as a given genotype is neither replicated nor tested in differing environments. Mass selection therefore always has confounding of environmental conditions that can mask genotypic differences. As breeders can only marginally influence the extent to which observed differences are determined by genetic causes (h = square root of heritability), mass selection is only effective for traits with higher heritability and little genotype by environment interaction.

One factor that can be better managed to increase response to selection (R) is the intensity of selection (i) used in mass selection. As the test units are single plants it is relatively easy to increase selection intensity by increasing the area sown with the population bulk, to have a greater number of plants from which to select the minimum number of desirable plants to constitute the next cycle.

The extent of additive genetic variation that can be exploited by mass selection depends on the level of parental control. If the trait can be evaluated before flowering and undesirable plants culled, full parental control can be imposed and the full extent of additive genetic variance can be exploited. For traits that can only be observed after flowering, only the female parent can be controlled, and thus only 50 percent of σ_A can be exploited (Table 12.1), unless plants are self-pollinated and the selfed progenies are used for recombination.

12.6.1 For which selection objectives and conditions can mass selection be useful?

Mass selection is a very simple method of selection, as selection is based on individual plants. This method thus requires minimal materials and organization for implementation. Mass selection enables maintaining a very large effective population size even with high selection intensity. Several thousand plants can be evaluated and several hundred retained to create the next cycle of the population. An additional advantage of mass selection is that each season results in the recombination among differing gene blocks in the population. This frequent recombination is essential for breaking undesirable linkages and increasing the frequency of desirable trait combinations. This is very important during the initial phases of a recurrent selection programme, when new parental materials are being recombined to form new populations, or when a new variety is formed from partially inbred progenies.

Mass selection will be most effective for traits that are highly heritable, with genetic differences that are observable on individual plants. One study in pearl millet showed quite acceptable heritabilities for single plant expression of plant height (0.58), seed weight (0.52) and flowering date (0.45), but not for grain yield (0.29), based on

parent-offspring regressions conducted in several populations (Rattunde, Witcombe and Singh, 1989). Thus mass selection for traits such as grain colour, grain size or form, plant height or time to flower can be effective, as these traits are expressed in a rather consistent manner, even with moderately heterogeneous soil conditions.

Mild selection with culling of undesirable types can be useful in newly created populations in which the introduction of new diversity or traits is accompanied by introduction of genes (or gene combinations) with undesired effects on quality or adaptation. This was the case in the early stages of the farmer-participatory population breeding work in Burkina Faso (Box 12.1). More intense selection can be applied when trying to concentrate favourable genes, for example with resistance to a pest or adaptation to specific conditions (as described in Box 12.2).

12.6.2 Potential roles and contributions of farmers

Mass selection is the method used by farmers for creating and maintaining the majority of the world's heritage of landrace varieties. Farmers are often skilled at single plant selection, with sophisticated mental indices for weighing several critical traits that are considered during selection, particularly for indigenous crops that they have developed over countless generations of selection. Sorghum farmers in Mali, for example, when choosing each panicle for use as seed consider several aspects of grain type (colour, size), glumes (ease of threshing) and panicle form (optimal density of grains and numbers of panicle branches, but with sufficient spacing to avoid risk of damage from insect feeding). Farmers may observe certain traits more accurately and with more practiced judgment than formal breeders, particularly for crops in their centres of origin or diversity. Likewise, farmers can weigh the importance of many traits, and set acceptable thresholds for each trait based on the importance of each to meeting their needs. Farmer mass selection also enables selection to be based on plant expression under their own field conditions. Involvement of farmers in mass selection also allows a larger scale of operation than would be possible for individual breeders, with possibilities of several farmers participating, each contributing their time and expertise to observe thousands of plants and select those showing most promise under their field conditions. Weltzien, vom Brocke and Rattunde (2005) propose options for farmer participation in mass selection.

12.6.3 Factors for success

The genetic gains achieved via mass selection can be maximized by attention to factors influencing the three components of the Selection Response Formula (see Chapter 2).

Heritability (h)

The appropriate choice of field and management of the field can help favour expression of genetic differences for the target trait(s). Pre-sowing observations of the terrain can help to chose sites where there is less soil heterogeneity, shading and nutrient effects of trees, piles of animal dung or residues from previous years. Likewise, the planning and uniform application of management practices should help favour expression of genetic differences for the desired target traits. Further, the standards for selection can be adjusted based on the apparent environmental conditions, relaxing standards in patches of poorer growth or raising standards in areas with exceptionally luxuriant growth. Gridded

BOX 12.1

Use of zone-specific sorghum populations as source material for variety development

Zone-specific broad-based sorghum populations were created to serve as sources of genetic diversity for deriving new varieties that combined increased grain productivity with the grain quality and adaptation of the farmer's own varieties for the Central-North (650 mm average annual rainfall), Central-West (800 mm) and Boucle de Mouhoun (900 mm) areas in Burkina Faso (vom Brocke *et al*., 2008). As the parental materials were of diverse Guinea- and Caudatum-race origins, farmers applied mild selection for grain quality during the back-crossing and recombination cycles to increase the probability of deriving useful segregates for variety development in the resulting populations.

The varietal development process began in each of the three zones by two farmers, one per village, sowing approximately 10 000 plants of the zone-specific population in isolated fields representative of the most important production system in the area. A group of 10 to 25 farmers, both women and men, selected panicles from the population bulk, with each farmer choosing about three of the most desirable panicles for the specific grain or plant type of most interest to them. A total of about 250 panicles were selected per site, and thus 400 to 600 plants per population were selected with a selection intensity of about 2 to 3 percent. Selection by several farmers and in different field environments helped to better sample the plant types to address farmer's different needs and provide a sufficiently large number of progenies for appropriately intense selection in subsequent generations.

The S_1 lines obtained from the selected S_0 panicles were prepared in sets according to the 'variety type' category for which they were selected, and single-replicate nurseries were sown by individual farmers. Selection among and within progenies was applied according to normal pedigree variety development methods.

The fate of progenies selected out of the 2004 Boucle de Mouhoun population for variety development are tabulated below.

Variety type (primary selection criterion)	2004		2005			2006
	S_0 plants selected by farmers	S_0 panicles (S_1 lines) retained by breeder	S_1 lines selected by farmers	Panicles ($S_{2.1}$ lines) selected by farmers in retained S_1 lines	S_2 lines retained by breeder	S_2 lines selected by farmers
Couscous	8	6	3	3	3	2
Malting and beer	34	24	6	12	9	-
Food qualty (tô)	40	28	11	16	14	-
Commercial grain	31	24	8	11	7	-
Grain storability	27	19	6	7	3	-
Fodder	50	30	12	15	14	3
New panicle type	46	32	4	4	3	2
Early maturity	55	36	6	12	7	2
Striga resistance	31	22	10	17	12	-
Stems (construction)	39	26	10	10	4	1
Total	**361**	**247**	**76**	**107**	**76**	**10**

(K. vom Brocke, G. Trouches, C. Barro-Kondombo and J. Chantereau)

BOX 12.2

Origin of a flooding-tolerant sorghum population

ICRISAT-Mali conducted several cycles of mass selection in a broad-based random-mating sorghum population with genetic male sterility to recover the special Guinea-race glume and grain characteristics required for free threshing, resistance to grain mould and desirable food quality. The field where this population was grown in 2001 was flooded for three weeks when the river rose due to unusually heavy rains. The more desirable plants that survived that year were selected as probably possessing some tolerance to water logging, as the entire field was flooded. The same year, farmers expressed interest in having a sorghum variety for fields that tend to be inundated in years of heavy rainfall. The following year this 'waterlogged' cycle bulk was given to two farmers in different villages, who sowed it in low-lying fields adjacent to their own sorghum variety. The farmers liked the population very much, and one of them, Diakaridia Dembele, started selecting panicles within it for use as seed the following year. The next year the population performed exceptionally well and he selected panicles for seed for himself, but he also gave away 75 kg of seed in response to demand from many neighbours. Most of the farmers requesting seed were women who grow rice in low-lying areas and used this new sorghum 'variety' on the borders of their fields, where risk of temporary inundation was high. The farmer planned to continue selection in this population for one or two more seasons to obtain an acceptable level of uniformity for glume colour and panicle form, at which time he could consider it to be a finished variety.

(E. Weltzien, D. Dembele, S. Diakite and F. Rattunde)

mass selection offers a systematic approach by dividing the field into grids, and selecting a common number of plants from within each grid.

The effectiveness of selection between plants can be maximized by ensuring that selection is conducted by the most skilled people. For example, the threshability (ease of separating grains and glumes) of sorghums in West Africa can be best observed by farmers who have years of experience and a cultural heritage of selection for this trait. Effectiveness of selection may be further raised by identifying individuals who are the most interested and locally respected for their capabilities as 'seed experts'.

The genetic gains from mass selection in out-crossing species can be increased through parental control that reduces the extent to which selected plants are pollinated by unselected plants. Self pollination and selection of selfed plants achieves maximum parental control. The same result is achieved with populations of self-pollinating species containing genetic male sterility, through identification and selection of male-fertile plants. Note however that selection of selfed plants would require a separate recombination to constitute the next cycle bulk. If introgression from neighbouring fields is not desired, sufficient isolation distance would need to be maintained. Culling undesired plants prior to flowering also provides parental control and could therefore double gains for traits that can be observed before flowering. Culling out

tall plants in a dwarf population is one such example.

Genetic Variance (σ_A)

The choice of parents for creating the initial population determines the level and usefulness of genetic diversity. The more diverse the parents chosen, the higher will be the expected genetic variance and therefore the potential gain from selection. There is usually an optimal level of diversity beyond which the mean performance of the population would go down, thereby reducing the usefulness of the population in the long-term (Schnell and Utz, 1975).

Maintaining sufficient population size through selection and recombination of a large enough number of plants will help maintain a desirable array of alleles, and assure genetic variation exists for selection in subsequent cycles (Witcombe and Virk, 2001). Likewise, maintaining a sufficiently large effective population size is indispensable to avoid inbreeding, which is of greatest concern in highly outcrossing species. It is exhibited as a loss of vigour of the population and undirected separation into distinctive lines due to random fixation of genes. Effects of inbreeding and strategies for avoidance are summarized by Allard (1999) and Hallauer and Miranda (1981), among others. Using a minimum of 200 plants to create the next cycle bulk will minimize loss of genetic variation and genetic drift that would otherwise arise from mating among a limited number of parents and sampling.

Mass selection, based on single plant selection in a given site, can produce more site-specific responses than would be obtained with multi-environment progeny testing. However, where the objective is to produce an improved population and eventual varieties with wider adaptation, a population may be grown by several farmers or researchers, with selections from differing sites being pooled by breeders to capture selections that represent a wider sample of conditions or selection criteria. Selections produced by different breeders on differing sites could be simply bulked, or they could be grown out in isolation for recombination, with eventual culling of certain off-type progenies.

Selection Intensity (i)

More intense selection (setting higher thresholds for retaining plants) is expected to increase the genetic gains. The selection intensity coefficient in the genetic gains formula corresponds to the number of standard deviations by which the mean of the selected fraction exceeds the population mean (Falconer, 1981; Becker, 1993), and thus depends on the percentage of selected individuals.

An advantage of mass selection is the possibility of achieving very high selection intensity, by which rare plants possessing the desired combination of several traits or express rare forms of a given trait can be identified. However, to realize this potential it is necessary that a sufficiently large number of plants be available for selection. For example, a population with 10 000 plants could be subjected to selection with a 2 percent selected fraction and still retain 200 plants for reconstituting the next cycle bulk. Observation of farmer selection in sorghum populations in Burkina Faso shows that they frequently retain selected fractions ranging from 0.2 to 5 percent.

12.7 PROGENY-BASED RECURRENT SELECTION METHODS

A range of methods for population improvement rely on testing, selecting and recombining families rather than individual

TABLE 12.2
Selection response per year for pearl millet head yield from alternative recurrent selection procedures using equal level of resources and optimized for allocation of labour

Recurrent selection procedure	Selection response
Mass selection(1)	0.22
Half-sib family(1)	0.34
Full-sib family	0.51
S_1 line (one stage)	0.27
S_2 line (one stage)	0.23
S_1 line (two stage)	0.26
S_1 line/S_2 line	0.26
Full-sib/S_1 line	0.46

Notes: (1) S_1 lines from S_0 single plants used for recombination.
Source: as presented in Schipprack, 1993.

plants. These progeny-based methods may involve a single stage of selection or multi-stage methods that combine evaluation and selection of genetically different evaluation units and selection units in successive seasons or generations (Hallauer and Miranda, 1981; Schipprack, 1993). Superior progenies identified through this testing can be used in a pedigree breeding programme to directly develop new inbred varieties, or to develop parental lines for production of hybrids or synthetics (Box 12.4). For highly outcrossing species, superior progenies of similar agronomic type and maturities could be used to create new open-pollinated varieties by random mating.

The objectives pursued with progeny-based recurrent selection methods tend to be improvement of traits whose expression is unreliable on a single-plant basis, and the development of superior progenies or inbred lines. Increasing yield is a typical objective pursued by progeny-based selection methods, where replicated trials conducted in multiple environments are used to determine the genetic potential of the evaluation units. Modelling of expected selection responses of alternative recurrent-selection methods for pearl millet grain yield show that certain progeny-based selection schemes may achieve twice the genetic gain for grain yield compared with mass selection (Table 12.2), even with comparable allocation of resources and optimized for labour use (Schipprack, 1993).

Factors for success

Questions to consider for maximizing the response to selection include how many traits are to be improved and when selection for specific traits is conducted during the inbreeding process. Each additional selection criterion will reduce the potential gain for the individual characters. Farmer indications of acceptable thresholds and priorities for specific traits can be helpful to focus selection efforts. Selection for traits with higher heritabilities is recommended when single plant selections are used to generate progenies or in early generations. In contrast, selection for less heritable traits is best conducted in later generations when multiple-environment assessments are feasible and progenies are more homozygous.

Farmer's assistance in single plant selection can be useful to funnel the most promising genetic materials into further stages of testing, and thus use limited testing resources most effectively. Farmers can help create the progenies used to initiate the selection procedure through mass selection of half-sib or S_1 lines from recombined bulks. They can further assist by selection within progenies in on-station or on-farm nurseries. Selection by several farmers helps to retain diversity, especially as cultivar preferences may differ with differing socio-economic backgrounds or production objectives.

Effective population size is also important for progeny-based selection methods. Initial progeny trials should consist of

at least 200 progenies that will allow an appropriate intensity of selection (15 to 30 percent) and still retain a sufficient number of progenies for recombination or a subsequent stage of selection. The initial creation of progenies (S_1, full-sib (FS) or half-sib (HS) for example) can thus be done by selection from thousands of plants. Mass selection by several farmers, each sowing the same bulk in their own fields, has been useful in achieving suitably large numbers of selected progenies.

Progeny evaluations conducted in sufficient test environments is also important to effectively assess genetic potential and to sample the environmental diversity. For example, the wide range of sowing dates, soil and rainfall conditions for sorghum production in even a single agro-ecological zone of West Africa requires a minimum of four to six test environments to provide some measure of representation. Conducting progeny trials on farmers' fields, although logistically challenging, can help achieve the necessary, and appropriate, sampling of test environments.

Progeny-based trials conducted with farmers presents several challenges not encountered with mass selection. The large number of progenies and more complicated trial designs requires researcher assistance, at least during planting and harvesting, or even researcher management of on-farm trials. Trial designs can be modified to make on-farm progeny testing feasible. Individual farmers could, for example, grow a single replication or even a subset (incomplete block) of test entries. Modern statistical procedures and computing power now make analysis of the widest range of incomplete and unbalanced designs possible. Issues of how benefits and costs are shared also need to be considered, since land and labour requirements may be much higher and direct benefits to participating farmers less than in the case of mass selection.

12.8 EVOLUTION OF POPULATION IMPROVEMENT PROGRAMMES

Although population improvement programmes can follow a single selection methodology for improvement of a given trait or set of traits over many cycles (Rattunde and Witcombe, 1993), this may not often be the case. Population improvement may begin by conducting several cycles of mass selection to narrow and 'clean up' the population to a more acceptable range for critical adaptation or quality traits. Populations may reach appropriate ranges for simply inherited traits after a few cycles of selection and little further progress will be made by selecting for these same traits. Improvement of more complexly-inherited traits, such as yield, would require changing to progeny-based selection methods.

Practical population improvement programmes can also undergo major changes in the breeding objectives in response to evolving needs and opportunities. For example, population improvement by ICRISAT-Mali was initially conducted on a sorghum population of tall plant height as this plant height corresponds to what most farmers grow in the target Sudanian zone of West Africa, and tall parental materials had the required suite of adaptive and quality characteristics. However, the convergence of farmers' priority setting that placed highest value on increased yields, the hypothesis that reducing heights could raise harvest index and thus grain yields, and the identification of novel dwarf segregants in the ongoing population improvement work, led to a major shift to dwarf population and variety development, as described in Box 12.3. Further, this sorghum population

BOX 12.3

The evolving Guinea-race sorghum populations in Mali

A broad-based sorghum population was developed as a source of diversity for breeding sorghum varieties with increased grain yield and the grain, glume and panicle characteristics required for adoption in the Sudanian zone of Senegal, Mali and Burkina Faso in West Africa. BC_1 or BC_2 progenies, created by crossing 13 higher yielding Guinea-landrace varieties to a source population segregating for the *ms3* genetic male-sterility gene locus, with subsequent backcrossing, were bulked together in 1994 (Rattunde *et al.*, 1997). Three cycles of recombination with mild selection and one cycle of more intense mass selection for grain and glume traits followed. Progeny-based selection was then initiated, using S_1 and S_2 progeny testing schemes, for increasing the population's yield level and to derive sorghum varieties with superior grain yield. This population and the varieties derived from it had plant heights of 3 to 5 m, similar to the landrace varieties used to create the population.

A new Dwarf Guinea Population was initiated in 1999 by selecting 50 plants with short stem-internodes (40 male-fertile and 10 male-sterile plants that gave $S_{0.1}$ and half-sib progenies, respectively) out of a total of 15 000 plants of the original tall Guinea Population These progenies were recombined together, as well as inter-mated with 12 dwarf progenies derived from previous population cycles and five short-statured inter-racial varieties produced by pedigree breeding. This new population was recombined, with the second cycle involving replicated randomized sowings of 240 F_1s to assure thorough recombination. The presence of desirable Guinea-race grain and panicle types on these markedly shorter plants (mean: 2.5 m) was confirmed by farmers, who identified approximately 200 superior S_0 plants.

Two hundred S_1 progenies, derived by selecting the most desirable S_0 male-fertile plants from the Dwarf Guinea Population, were tested in a replicated yield trial and selfed in a separate nursery to advance to the S_2 generation. A total 70 S_2 progenies from the highest yielding S_1 progenies were further evaluated for yield at the ICRISAT-Samanko station (two dates of sowing) and the IER-Kolombada (Mali) station the following year (2003). A total of 20 selected progenies were then recombined to create the cycle 2 bulk of the Dwarf Guinea Population. The recombination was conducted by first making paired crosses among progenies, and the following year random-mating in isolation of all crosses.

(F. Rattunde, E. Weltzien, A. Toure, J. Chantereau and C. Luce)

breeding programme will continue to adjust towards the emerging needs of dual-purpose (grain+fodder) varieties and of new short-statured lines as hybrid parents.

An even more rapid evolution of populations can occur in certain crops, like highly outcrossing pearl millet, where relatively few cycles of improvement are conducted between periodic crossing between populations (Rattunde *et al.,* 1997). This approach would require working with a number of different populations, and possible structured, diallel, population crossing to identify the most promising populations for continued improvement. By periodic inter-population crossing, heterosis could

BOX 12.4

Lata (Bala Berthe): A dwarf Guinea-race sorghum variety and hybrid parent developed through population breeding

S_3 progenies (n = 89) derived from the most promising S_2 progenies (see Box 12.3) were further tested in replicated, multi-environment, on-station trials for variety development. Six of the most promising progenies were included in a 16-entry, 2-replicate, early generation variety trial conducted by 20 farmers in 10 villages in 2005. Each entry was given a name to facilitate discussions by farmers. The progeny 'Lata' showed higher yield, intermediate height (2.5 m), and was appreciated by farmers. This progeny was given to Bala Berthe, a farmer with strong interest and expertise in selection. Bala Berthe conducted two cycles of mass selection for panicle architecture, grain and glume characteristics and shared a portion of his seed lot with ICRISAT. The variety Lata (Bala Berthe), following further testing in larger scale 4-entry 'Variety Test Kits', was submitted for variety registration in Mali. This variety was also used as a male parent to produce a series of experimental hybrids. Analysis of multi-environment hybrid yield trials showed that Lata (Bala Berthe) had the highest combining ability of all male parents in 2007.

be exploited, increasing the mean productivity as well as the genetic variation of the resulting inter-pool populations.

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CHAPTER 13

Selection methods Part 5: Breeding clonally propagated crops

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13.1 INTRODUCTION

The literature about participatory breeding of clonally propagated crops is very limited. This makes it difficult to write this chapter exclusively about how farmers have bred, how they breed—with or without support of scientists—and how they should breed clonally propagated crops. There are today clear definitions for participatory plant breeding (PPB), participatory variety selection (PVS) (see Chapter 9) and indigenous plant breeding (IPB; the selection process of farmers for more than 60 centuries). Breeding by scientists and economic entities has been redefined as formal plant breeding (FPB), consisting of breeding carried out on-station, linked with multi-location trials, and assisted by quantitative genetics, selection theory and biotechnology (with or without recombinant DNA technology). A variety developed through FPB is termed a modern variety (MV), in contrast to a farmer-bred variety (FV), which is developed by IPB. Especially in clonally propagated crops, FVs continue to dominate crop production in many developing world regions. Obviously FPB has not been so successful, because farmers decided to continue to grow FVs instead of adopting MVs (Friis-Hansen, 1992; Witcombe *et al.,* 1996). Definitions are helpful, providing a common term for the same technique or method. Here we need to bear in mind two points when considering such definitions and the issues associated with them:

- the every-day formula used to predict the response to selection (with all its extensions to several selection steps and traits) may well be the most useful tool given to breeding by statistics; and
- breeders have to adapt a crop to human needs and they must pay adequate attention to the needs of clients.

13.2 AN OVERVIEW OF CLONALLY PROPAGATED CROPS

What are clonally propagated crops?

Standard textbooks list a surprisingly large number of crops: all important root and tuber crops, many forage crops, nearly all types of fruit and wooden ornamentals, many cut flowers and pot plants, as well as forest trees. The definition of a clonally propagated crop is that the material to cultivate and maintain a variety is obtained by asexual reproduction, regardless of how different the plant material used for propagation is within and between species, encompassing tubers, roots, stem cuttings and corms, as well as asexually developed seeds (seeds developed without meiosis). It should be remembered that if crops such as maize (bred as an open-pollinated or hybrid crop) or beans (bred as a cross-fertilized, self-fertilized or hybrid crop) were to be propagated by stem cuttings or asexually developed seeds, they would be clonally propagated crops. In contrast, in breeding clonally propagated crops, the breeding techniques and methods that are usually associated with cross-fertilized and hybrid crops can be very useful. An example is the selection of parents in potato, cassava and sweet potato breeding, which are recombined in open-pollinated polycross nurseries to create new genetic variation. It is almost certain that techniques and methods from breeding cross-fertilized and hybrid crops will become much more important in the future of clone breeding.

What is the general principle in breeding clonally propagated crops?

It appears to be simple: to break the normal clonal propagation by a crossing step, and thus develop sexual seeds and genetic variation from which to select new clones. All propagation steps from the first to the last

selection step are again 'normal' asexual reproduction (Simmonds, 1979). Hence, the finally selected clone is genetically identical with the original seed plant from which the selected clone is derived. In other words, each seed plant is a potential variety. Roots and tubers, fruit and tree plant species have been used by human since long before the dawn of agriculture. They have been domesticated by IPB (Simmonds, 1979) and several made a substantial yield progress by FPB in some regions of the world. However, in other regions of the world there is not much yield progress, and in these regions there appears to be a clear need of PPB for progress. We wish to illustrate this by two examples: potato and sweet potato.

An example of the needs and requirements of clonally propagated varieties can be found in potato (*Solanum* spp.). There are about 200 wild potato species (Huamán and Ross, 1985). They usually contain glycoalkaloids, which give tubers a bitter taste and which are toxic when consumed in large quantities (Zitnak and Filadelfi, 1985). It is nearly certain that 100 to 130 centuries ago indigenous knowledge in the Andes and along the Pacific coast of South America was those sites where it was possible to collect wild potato tubers where species and mutants were growing that had low alkaloid content. Although these tubers were very small, the man, or more probably a woman, made life much easier by growing and maintaining desirable types by cloning close to their homes. This happened more than 8 000 years ago, and most likely independently at several places (Ugent, Pozorski and Pozorski, 1982; Ugent, Dillehay and Ramirez, 1987). Those types were preferred that were easier to maintain, easier to harvest (shorter stolons) and had larger tubers compared to other types. The result was the domestication of *pitiquiña* (*Solanum stenotomum*), which was most probably selected from *S. leptophyes* or *S. canasense*. From the view-point of the knowledge of the twenty-first century it is not surprising that suddenly potato plants with larger leaves and larger tubers were found. Potato spontaneously changes its polyploidy level by unreduced gametes and recombination. Polyploid potatoes are more vigorous than their diploid ancestors. The result was the domesticated of polyploid *andigena* (*S. tuberosum* subsp. *andigena*). Andigena is the ancestor of the commercial potato in long-day temperate climates—the so-called *Irish potato* (*S. tuberosum* subsp. *tuberosum*) (Hawkes, 1979, 1981). This IPB of potato and introductions of FV of potato into the Northern Hemisphere changed the world both socio-economically and politically (Hobhouse, 1985).

Today, eight species of potato are still cultivated in the Andes, variously diploid, triploid, tetraploid and pentaploid:

(i) cultivated diploid potatoes are pitiquiña (*S. stenotomum)*, its close relatives *phureja* (*S. phureja)* and *limeña* (*S. goniocalyx*), and *ajanhuiri* (*S. ajanhuiri*), which evolved from interspecific recombination of diploid pitiquiña and the diploid wild potato species *S. megistacrolobum*;

(ii) cultivated triploid potatoes are *chaucha* (*Solanum* × *chaucha*), a hybrid between diploid pitiquiña and tetraploid andigena, and *rucki* (*Solanum* × *juzepczukii*), a hybrid between diploid pitiquiña and the tetraploid wild potato species *S. acaule;*

(iii) cultivated tetraploid potatoes are andigena and Irish potato; and finally

(iv) the cultivated pentaploid potato is a hybrid species (*Solanum* ×

curtilobum), which evolved between tetraploid andigena and triploid rucki, and unfortunately is also called rucki (Hawkes, 1981; NRC, 1989).

The andigena is the best known potato in the Andes (with about 2 500 known FVs). It is cultivated in tropical mid-elevation valleys and mountainsides. The second most important potato is phureja, which is cultivated on the warm and moist eastern slopes of the Andes (with about 500 known FVs), followed by *limeña*, ajanhuri and rucki. Limeña or *papa amarilla* is grown in the temperate areas of the Andes and still achieves high market prices due to its taste and flavour. Ajanhuri and rucki are the most frost resistant cultivated potato species and cultivated up 4 200 masl. The former is used as an insurance crop in cases where andigena fails due to unpredictable hail and frost (some ajanhuri varieties are bitter and must be processed). The latter are usually only eaten after having been processed into *chuño*, the famous storable food product of the Incas. Many of these potatoes have clearly better taste and flavour compared with what is considered potato in the Northern Hemisphere (Huamán 1983; NRC, 1989; De Haan, 2009). However, taste is a variable characteristic; it changes from person to person, from family to family, and from society to society. Moreover, many of these IPB potatoes are clearly superior in protein and micronutrient concentration in their tubers (pro-vitamin A, calcium, magnesium, iron and zinc) compared with MVs (Ochoa, 1990; Morris *et al.,* 2004; Burgos *et al*., 2008), and are useful as genetic resources or directly as FVs to alleviate malnutrition in the mountain regions of the world.

The potato and the Andes were chosen as an example to give an impression of an aspect of breeding that is as least as important as taste, flavour and nutrient content, namely the importance of adaptation of a crop and its varieties to the local environment. They who know the Andes also know that is unrealistic to breed a widely adapted potato variety for this region of the world. Temperature, rainfall, soil conditions (including salinity and drought) and pest and disease pressures change from microclimate to microclimate from sea level at the Pacific coast up to 3 500 to 4 500 masl in the Andean highlands (mid-elevation valleys and plateaus), and again down into the warm tropics, where the Andes meet the Amazon. Breeding potatoes in this region of the world was and can only be successful by decentralization and with farmer participation (Johns and Keen, 1986; Gabriel and Torrez, 2000). Admittedly this is an extreme example, but such situations can be found in less extreme form in nearly all regions of the world. In the Southern and Northern Hemispheres, potatoes generally must be day-neutral; in South-west and Central Asia, potatoes must be very quick to mature, with a short crop duration of 80 to 90 days; in Europe and Northern America, more than 30 quality characteristics combine to determine tuber quality for market needs; and finally in the UK, a potato variety must be white fleshed, whereas in Germany it must be yellow fleshed, otherwise it is not eaten (CIP, 1984; Levy, 1984; Tarn *et al*., 1992). An additional major factor for adaptation and acceptance of potato varieties is their tolerance and resistance to diseases and pest. In all temperate and moist climates, potato farmers have to fear Late blight (*Phytophthora infestans*), which is not important at temperature above 25°C, but then Early blight (*Alternaria solani*) takes over. In tropical lowlands, the farmer has to fear Bacterial wilt (*Pseudomonas solanacearum*), and in all warm dry regions

the potato crop can be lost because of Colorado beetle (*Leptinotaras decemlineata*) (CIP, 1977, 1980; Radcliffe, 1982; Rich, 1983).

A simpler example for the needs and requirements of clonally propagated varieties can be found in sweet potato (*Ipomoea batatas*). Sweet potato was domesticated in the Americas more or less during the same prehistoric period as the potato (O'Brien, 1972). The evolution of sweet potato was not as complex and diverse as that of potato. There are about 500 *Ipomoea* species, but only the *I. batatas* species was domesticated (Austin and Huamán, 1996). Again polyploidy was important. Sweet potato is hexaploid and its closest relative is *I. trifida* (di- and tetraploid). It is certain that sweet potato contains the *I. trifida* genome, but obviously it is not simply a multiple copy. Two-thirds of the sweet potato genome corresponds to the *I. trifida* genome and one-third to an ancestor very closely related to *I. trifida* (Shiotani and Kawase, 1989). Within diploid *I. trifida* accessions (seed families) it is also possible to find plants that form small storage roots (Daniel Reynoso, pers. comm.). However, sweet potato has been found in the ruins of the so-far oldest city in the Americas, *Caral* on the Pacific coast of central Peru (Solis, 2004), and the crop reached Pacific Polynesia and parts of South-East Asia (naturally or by early seafarers) before Columbus. It was primarily the Portuguese that introduced it into Europe, Africa, South Asia and East and South-East Asia (Yen, 1976).

Although the taste of sweet potato in FVs and MVs differ tremendously, two major types can be distinguished: (i) the orange-fleshed, moist, low dry matter (DM) and sweet type, which has a soft mouth feeling; and (ii) the white- or pale-yellow-fleshed, high DM, low-sweet or bland type, which has a dry mouth feeling. The first type, also called the dessert type, has extremely high pro-vitamin A concentrations (Huang, Tanudjaja and Lum, 1999) and a 50 g piece of fresh storage roots can meet the daily requirements of a pre-schooler (Low *et al.*, 2007). Moreover, sweet potatoes with high pro-vitamin A concentrations have high protein and mineral concentrations (Grüneberg, unpublished). In the United States of America, the dessert type is generally the desired sweet potato to meet market and consumer needs. In the Caribbean, low DM orange-fleshed sweet potatoes (OFSP) are consumed, but as a staple, so a dryer mouth feel and less sweet flavour is preferred. These white- or yellow-fleshed varieties are known as *bonitos* or *ricos* (Baynes, 1972). Along the Pacific coast of South America we observed that sweet potatoes are mainly pale orange fleshed and less sweet. However, locally, white- and purple-fleshed sweet potatoes are consumed, which clearly have different taste, texture and flavour compared to OFSP. In Brazil, the sweet potato storage roots must clearly have a high DM concentration (28 to 30 percent DM), and usually this is a white-fleshed sweet potato; however, locally, high DM OFSP can be found (Amauri Buso, pers. comm.).

The taste preferences in sub-Saharan Africa are similar to those in Brazil, perhaps because the Portuguese introduced the sweet potato into Africa. All FVs are nearly exclusively white- or yellow-fleshed and have high DM concentrations; however, a few pale- to medium-orange-fleshed FVs can be found with high DM concentrations (Tumwegamire, unpublished). These local OFSP FVs are very promising for alleviation of vitamin A deficiency in sub-Saharan Africa (e.g. FVs such as 'Ejumula', 'Carrot

C', 'Carrot Dar es Salaam' or 'Zambezi'). In eastern Africa, storage root DM contents must be >30 percent (Mwanga *et al.,* 2003). In southern Africa, storage DM concentration between 26 and 29 percent are accepted (Laurie Sunette, pers. comm.), whereas in West Africa, sweet potato must be non-sweet, very high in DM concentrations (between 30 and 35 percent DM) and with a texture and flavour tentatively similar to yam (*Dioscorea* spp.) (IITA, 1981). In contrast, in India, where sweet potato consumption has been very low in the past, today people prefer sweet potatoes with high DM, high sugar content, dark orange flesh and a storage root shape that is cylindrical but tapering at both ends (Sreekanth Attaluri, pers. comm.).

In addition to regional and local preferences for storage-root colour, DM, texture and taste, the acceptability of sweet potato varieties is mainly determined by pest and disease pressures. However, the number of pest and diseases in sweet potato are considerable lower than in potato. Generally, sweet potato varieties must have a certain degree of tolerance to Sweet potato virus disease (SPVD). The disease occurs after infection by two viruses: the Sweet potato feathery mottle virus (SPFMV) and the Sweet potato chlorotic stunt virus (SPCSV). The SPCSV is the more problematic component of SPVD, because yield losses due to SPFMV, in the absence of SPCSV co-infection, are low and SPFMV resistance in sweet potato breaks after the plant is infected by SPCSV (Gibson *et al.,* 1998; Karyeija *et al.,* 2000). SPVD often causes serious yield losses in high-virus-pressure zones of sub-Saharan Africa, and American OFSPs have failed in many regions of sub-Saharan Africa due to insufficient SPVD tolerance. Although the virus pressure of SPVD along the Pacific coast of South America is not extreme, farmers have not adopted MVs (e.g. cv. INA100, which is a high yielding OFSP and fits consumer needs very well), because of insufficient SPVD tolerance. Farmers became disappointed with new MVs and after a few growing seasons returned to FVs such as cv. Jonathan and cv. Huambachero. OFSPs from the Americas with elevated DM (e.g. cv. Jonathan) are partially successful in southern Africa, and in south-west and central Asia, provided that weevil pressure is not extreme. Weevil damage is associated with drought-prone regions (Central and South America, sub-Saharan Africa and south-west and central Asia); however, weevil species differ: *Cylas formicarius* in all parts of the tropics, *C. puncticollis* additionally in Africa, and *Euscepes postfasciatus* in the West Indies. On-station and farmers' field experiments show that there are significant differences in weevil tolerance among sweet potato genotypes (Hahn and Leuschner, 1981), but this tolerance appears to be less pronounced or inexistent on-farm. At the same time, farmers in drought-prone regions of Malawi want sweet potatoes in which storage roots are formed deep in the soil and which are clearly tapering at the top, because they associate this with less weevil damage (Ibrahim Benesi, pers. comm.). Moreover, latex in the storage root skin has been associated with less weevil damage by farmers, and varieties like Santo Amaro from Brazil clearly have considerably less weevil damage than other sweet potato varieties (Rafael Vasquez Martinez, pers. comm.).

The International Potato Center (CIP) is promoting OFSPs to alleviate vitamin A deficiency in the world (Low *et al.,* 2007; Pfeiffer and McClafferty, 2007). However, introductions from the Americas failed in the high-SPVD-pressure zone of eastern Africa (as did the FV Jonathan). To a certain extent this was associated with the

storage root flesh colour and taste. At the same time, local African OFSP FVs, such as Ejumula, Carrot C, Carrot Dar es Salaam and Zambezi, and locally-bred OFSP MVs, such as NASPOT5 (Mwanga *et al.,* 2003), have been adopted after awareness campaigns on the vitamin A deficiency problem (Regina Kapinga, pers. comm.). For this reason, CIP puts emphasis on decentralized sweet potato breeding, and has recently started to recommend incorporation of at least one participatory selection step in the breeding process.

In sweet potato breeding for human consumption, decentralization is characterized by a general overall goal: that of developing more OFSP varieties that meet local needs and consumer preferences, to alleviate hunger and malnutrition and to improve public health. The emphasis is on organizing OFSP breeding in eastern and southern Africa, with national OFSP breeding programmes starting recently in West Africa (Ghana and Nigeria) and south-west and central Asia (India, Bangladesh and Sri Lanka). Breeding is almost exclusively carried out by national agricultural research system (NARS) breeders and on NARS breeding stations, with currently 12 NARS and two sweet potato breeders from the CGIAR system involved. NARS breeders are provided with funds for parental recombination and to consider the quality trait of storage root flesh colour in the breeding process. Main emphasis in breeding is given to: (1) material exchange at the seed and clone level, (2) exchange of information, knowledge and results from breeding trials by annual meetings, reports and back-stop visits, and (3) a sweet potato breeding research and training build up on the needs shaped among discussions between NARS and CGIAR breeders. This has resulted in an additional aim to build up regional platforms for sweet potato breeding in eastern, southern and West Africa, with a focus on dual purpose OFSP (human consumption and animal feed), drought tolerant OFSP, and non-sweet high DM OFSP. PPB has so far mainly been a research component in the organization of sweet potato breeding.

There are strong indications that PPB in early selection steps of the sweet potato breeding process increases the efficiency and minimizes the risk of making wrong selection decisions. In contrast to PPB, PVS is tentatively a form of on-farm evaluation (in the frame of a larger number of multi-location trials) and cannot be as efficient as PPB, because there is considerably less genetic variation, and, for highly heritable traits, there is nearly no genetic variation at later breeding stages among clones. Not surprisingly, akin to the role of IPB in potato crop evolution, it has been shown that farmers have the ability to manage selection stages in sweet potato (Gibson *et al.,* 2008). This is consistent with results for potato (Gabriel and Torrez, 2000) and cassava (Manu-Aduening *et al.,* 2006). Farmer selections are mainly made by visual screening. This includes quality characteristics, diseases and pests, as well as the growth type, which is to a certain extent associated with drought adaptation in sweet potato (see below on selection in early breeding stages). Most importantly, farmers use more criteria and characters to select sweet potato clones than do breeders in FPB (Gibson *et al.,* 2008). In such a situation, the risk of FPB is to ignore characters that are important for good overall performance of a clonal variety. However, in the study of Gibson *et al.* (2008) in three provinces in Uganda, the most important characteristics for selection by farmers in early selection stages were common to those used by

breeders, as were their relative weighting of characters, namely: (i) good root yield and big roots > (ii) SPVD tolerance or resistance > (iii) tolerance to drought, attractive root colour prior to cooking, straight root shape and orange- or yellow-flesh storage-root colour, and finally > (iv) tolerance to weevils. Characters of storage roots after cooking were not determined. It should be noted that in later selection stages (FVS) the priority list of farmers or relative character weights changed, namely: (i) good root yield, big roots, drought tolerance, sweet and mealy roots after cooking > (ii) early root maturity, continuous root yield for piecemeal harvesting, and weevil tolerance > (iii) long root storage in the soil, extensive foliage, tolerance to caterpillars (*Acraea acerata*), marketability, attractive colour of storage roots prior to cooking, and non-fibrous roots after cooking > (iv) followed by a group of characteristics with very low weights, such as good root yield in poor soils, good vine establishment, tolerance to rats and other vertebrates, non-sappy and no loss of taste in storage roots prior to cooking, soft texture, nice looking at the table, nice flavour and easy or quick to cook storage roots. For some characters (mainly biotic pressures, i.e. SPVD and weevil tolerance; Gibson *et al.,* 2008) there were clearly different weights given to characters in different provinces, which might reflect local biotic challenge. Moreover, farmers used more attributes (51 attributes) than scientists and breeders (11 attributes) to describe and distinguish varieties. To what extent this is important is not clear; however, it might show the importance to farmers of distinguishing varieties.

To summarize:

(i) not surprisingly, farmers have the ability to select successfully both in the early and later breeding stages of a breeding programme (Gabriel and Torrez, 2000; Manu-Aduening *et al.,* 2006; Gibson *et al.,* 2008);

(ii) selection by farmers, mainly by visual screening, is more efficient in earlier stages than in later stages of the breeding programme, which can also be explained by the larger genetic variation in early selection stages compared with later stages in breeding clonally propagated crops; and

(iii) so far, selection by farmers at early selection stages has only been applied to a sample of the genetic variation generated by FPB in crossing programmes and it must be more efficient to expose the full genetic variation to farmer selection in the breeding process.

In Sections 13.2 and 13.3 we suggest how this can be done in a cost-efficient way and without losing time in the breeding process. However, doubts remain as to whether farmers can efficiently use and treat large amounts of true seeds and true seed plants, which often appear in quite different amounts per cross combination and have quite different performance compared with plants grown from vegetative plant parts. It might be more useful that plant breeders germinate seeds and multiply for each family a reasonable numbers of clones so that farmers can select clones in small plots comprising a few plants (2 to 4 per genotype). A further advantage of this is that the breeder can use the frequency of selected clones per family by the farmer as additional information to identify appropriate parents for recombination. However, we think that the selection of parents in breeding clonally propagated crops should have a participatory component, but should be mainly carried out by the breeder due to the genetics (see Section 13.2) and statistics

(see Section 13.5) involved in appropriate choices of parents in breeding clonally propagated crops.

To consider the range of needs, preferences and adaptation requirements for the large number of clonally propagated crops is out of scope in this chapter. Here we want to give the principles of breeding clonally propagated crops and how PPB can be carried out or linked into these breeding programmes. The breeding objectives and methods will be considered for four agricultural crops in more detail at the end of this chapter, namely: potato, sweet potato, cassava, and banana or plantain. Table 13.1 gives the plant parts used for propagation, the world production and the area harvested, as well as the polyploid level of the most important clonally propagated crops in agriculture. Obviously, quality characteristics determined by consumer preferences and market needs are key characteristics for breeding clonally propagated crops, because many of these are eaten fresh, or are only boiled or roasted, and when they are processed this is often carried out at the household level. Exceptions are sugar cane, fruit crops used for the juice industry, and to certain extent root and tuber crops (potato, cassava and sweet potato) when they are used for the starch, alcohol or biofuel industries. In resource-poor environments, yields and yield stability with low input are a priority, in addition to consumer acceptability. As has been mentioned above, a major factor that determines yields, yield stability and adaptation are pests and diseases. The most important pests and diseases of important clonally propagated crops in agriculture by eco-geographical region are given in Table 13.2, together with the most important quality characteristics.

Most clonally propagated crops are polyploid (Table 13.1). An exception is cassava, which can be considered as a polyploid behaving like a diploid (see below). Polyploidy is an important aspect

TABLE 13.1
Data on the 11 most important clonally propagated crops on a global basis

Species	Planting material	World production†	Area harvested†	Polyploidy
Potato (*Solanum tuberosum*)	Sprout tubers	315 $\times 10^6$ t	18.8 $\times 10^6$ ha	2x, 3x, 4x, 5x
Cassava (*Manihot esculenta*)	Hardwood cuttings	226 $\times 10^6$ t	18.6 $\times 10^6$ ha	2x
Sweet potato (*Ipomoea batatas*)	Sprout cuttings	124 $\times 10^6$ t	9 $\times 10^6$ ha	6x
Yam (*Dioscorea* spp.)	Root tubers	51 $\times 10^6$ t	4.6 $\times 10^6$ ha	3x–10x
Taro (*Colocasia esculenta*)	Corms	12 $\times 10^6$ t	1.8 $\times 10^6$ ha	4x
Sugar cane (*Saccharum officinarum*)	Cane stalks	194 $\times 10^6$ t ‡	20.4 $\times 10^6$ ha	8x
Banana and Plantain (*Musa* × *paradisiaca*)	Corms	105 $\times 10^6$ t	9.6 $\times 10^6$ ha	3x
Citrus fruit (*Citrus* spp.)	Bud stick grafting on rootstocks	89 $\times 10^6$ t	5.6 $\times 10^6$ ha	2x, 3x+1, 4x-3
Grapes (*Vitis vinifera*)	Hardwood cuttings	69 $\times 10^6$ t	7.4 $\times 10^6$ ha	6x
Apple (*Malus pumila*)	Bud stick grafting on rootstocks	64 $\times 10^6$ t	4.8 $\times 10^6$ ha	2x, 3x
Strawberry (*Fragaria grandiflora*)	Adventitious shoots	4 $\times 10^6$ t	0.26 $\times 10^6$ ha	8x

NOTES: † FAOStat 2006 at faostat.fao.org, ‡ Sucrose production.

TABLE 13.2
Quality characteristics, pests and diseases by production zone of the 11 most important clonally propagated crops in agriculture and horticulture

Major production zones	Quality characteristics	Pest and diseases
	Potato (*Solanum tuberosum*)	
Tropical highlands	Various fresh consumption traits, high iron and zinc contents, adaptation to various micro-climates	Late blight (*Phytophthora infestans*), cutworms (*Agrotis* spp.), potato tuber moth (*Phthorimaea* spp.)
Tropical lowlands	More uniform fresh consumption traits, high iron and zinc contents, extremely short crop duration (<80 days)	Bacterial wilt (*Pseudomonas* spp.), Early blight (*Alternaria solani*), Root-knot nematode (*Meloidogyne* spp.), viruses (Potato leaf roll virus (PLRV), Potato virus Y (PVY), etc.), year round aphid pressure
Temperate zones	Various fresh consumption traits, high starch for industrial use, various processing traits (chips, French fries)	Late blight, cyst-forming nematodes, (*Globodera* spp.), potato virus diseases (PLRV, PVY, PVX, etc.)
	Cassava (*Manihot esculenta*)	
Humid tropics	Cooking quality, elevated provitamin A content for human consumption with low HCN content, high DM for industrial uses	Bacterial blight (*Xanthomonas axonopodis*) in Asia, Africa and the Americas, Frogskin disease (CFSD) in the Americas
Drought-prone tropics	Cooking and processing (fried cassava, gari, fufu) quality, elevated provitamin A content for human consumption with low HCN content, high DM for industrial uses	African cassava mosaic (CMD) virus and Cassava brown streak disease (CBSD) in Africa, Green mite (*Mononychellus tanajoa*) and mealybugs (*Phenacoccus* spp.) in Africa and the Americas
	Sweet potato (*Ipomoea batatas*)	
Humid tropics	High DM WFSP and OFSP	Extreme Sweet potato virus disease (SPVD), especially in eastern Africa
Drought-prone tropics	Elevated DM WFSP and OFSP, and clearly non-sweet in West Africa	Sweet potato weevils (*Cylas* spp.) and SPVD to a lesser extent
Tropical highlands	Elevated DM	*Alternaria* spp. and SPVD to a lesser extent
Temperate zones	OFSP with low DM and WFSP with high DM (both with medium sugar content)	Root-knot nematode (*Meloidogyne* spp.) and SPVD to a lesser extent
	Yam (*Dioscorea* spp.)	
Humid tropics	Thirteen species with regional importance (main species *D. rotundata*), majority in wet hot tropics, but *D. abyssinica*, *D. alata* and *D. esculenta* also in dryer regions due to dormancy of tubers; growing time and taste varies extremely among species (some are poisonous and must be cooked)	Yam tuber beetles (*Heteroligus* spp.) and Anthracnose (*Colletotrichum* spp.), especially in West Africa, Yam nematode (*Scutellonema bradys*), Root-knot nematode (*Meloidogyne* spp.) and Shoe string virus disease
	Taro (*Colocasia esculenta*)	
Humid tropics	*Colocasia* cultivar groups: (1) one large corm with few cormels; and (2) several small cormels. Genotypes have very different shelf lives (dormancy period) and some require excessive processing before edible	Corm and root rots (caused by *Pythium* spp., *Phytophthora* spp., *Rhizoctonia* spp. and *Erwinia* spp.) and Dasheen mosaic virus (DMV) across world regions, Taro blight (*Phytophthora colocasiae*) and Taro beetle (*Papuana* spp.), especially in the South Pacific
	Sugar cane (*Saccharum officinarum*)	
All regions	Weight of canes, sugar content, juice purity, short or long vegetative times and adaptation to photoperiod (non-flowering)	In the past, virus diseases were most important; today they play a subordinate role due to resistance breeding and virus-free planting materials Pineapple disease (*Ceratocystis paradoxa*), Red rot (*Colletotrichum falcatum*), Smut (*Ustilago citaminea*), Shoot and Internode Borer (*Chilo* spp.) in nearly all regions
Humid tropics		Yellow leaf spot (*Cercospora* spp.), Scale insect (*Melanaspis glomerata*), Pyrilla (*Pyrilla purpusilla*)
Drought-prone tropics and subtropics		Eye spot (*Drechslera sacchari*), Whitefly (*Aleurolobus barodensis*)
Tropical highlands		Leaf scald (*Xanthomonas albileneans*), Wilt (*Cephalosporium sacchari*)

Major production zones	Quality characteristics	Pest and diseases
	Banana and plantain (*Musa x paradisiaca*)	
Humid tropics and subtropics	Bananas have a lower DM and higher sugar contents (very narrow genetic variation in triploid gene pool – ca. 30 cvs.) compared with high DM and starchy plantains (larger genetic variation in triploid gene pool – ca. 125 cvs.). Plantains are important staples in Central Africa and some parts of South America.	Banana wilt (*Fusarium oxysporum*) especially in the Americas, Yellow sigatoka (*Mycosphaerella musicola*), Black sigatoka (*M. fijiensis*) especially in Asia, Moko disease (*Pseudomonas solanacearum*), Bunchy top virus, nematodes such as *Radopholus similes*, banana root borer (*Cosmopolites sordidus*)
	Citrus fruit (*Citrus* spp.). Cultivated citrus may be derived from as few as four species: Key Lime (*C. aurantifolia*), Pomelo (*C. maxima*), Citron (*C. medica*) and Mandarin (*C. reticulata*). All other "species" are hybrids	
All regions	Very different tastes and fruit sizes (oranges — ca. 1100 cvs.; mandarins, lemons, pomelo). Citrus trees hybridize very readily and new hybrids easily maintained by apomixis.	Strong rootstock influence (*C. jambhiri, C. reshni, Poncirus trifoliata*) in adaptation to cold and resistance to *Phytophthora* root rot and virus diseases such as Tristeza, Porosis and Exocortis
Subtropics		Citrus canker (*Xanthomonas citri*), Foot rot (*Phytophthora* spp.), Melanose (*Diaporthe citri*), Blue and green mould (*Penicillium* spp.), Tristeza virus, nematodes such as *Tylenchulus semipenetrans*, fruit fly (*Bactrocera* spp.)
Drought-prone tropics		Foot rot (*Phytophthora* spp.), Gummosis (*Phytophthora* spp.), Citrus scab (*Elsinoe fawcetti*), Tristeza virus, Porosis viruses, fruit fly (*Bactrocera* spp.), citrus psyllid (*Diaphorina citri*), moth species such as *Ophideres, Sphingomorpha,* etc.
	Grapes (*Vitis vinifera*) North American species: *V. aestivalis, V. labrusca, V. rotundifolia*	
Temperate zones	The North American species are of interest for the summer rainfall regions in the tropics because of their disease resistance and minimal chilling requirement – especially in crosses with *V. vinifera* (better taste, better texture of berries)	Bunch rot (*Botrytis cinerea*), Downy mildew (*Peronospora sparsa*), Powdery mildew (*Erysiphe necator*), vine moths (*Eupoecilia ambiguella, Lobesia botrana*), eriophyid mite (*Calepitrimerus vitis*)
Drought-prone tropics and subtropics		Downy mildew, powdery mildew, Anthracnose (*Elsinoe ampelia*), beetles such as *Popillia japonica*, thrips (*Scirtothrips dorsalis, Thrips hawaiiensis* and *Rhipiphorothrips cruentatus*), grape root borer (*Vitacea polistiformis*), bugs such as *Lygocoris inconspicuous*, Grape mealybug (*Pseudococcus maritimus*)
	Apple (*Malus pumila*)	
Temperate zones	There are large differences in vernalization need among cultivars and several can be grown very successful in Mediterranean climates. Some cultivars in higher places in the equatorial region if the leaves are removed before beginning of bud dormancy (stripping off, or chemical defoliation)	Fireblight (*Erwina amylovora*), Apple rust (*Gymnosporangium* spp.), Apple scab (*Venturia inaequalis*), Plum curculio (*Conotrachelus nenuphar*), Apple maggot (*Rhagoletis pomonella*), Codling moth (*Cydia pomonella*)
Subtropics		Apple scab, Powdery mildew (*Podosphaera leucotricha*), Crown rot (*Phytophthora cactorum*), Apple crown gall (*Agrobacterium tumefaciens*), Bitter rot (*Glomerella cingulata*), root rots (*Phytophthora* spp.), Woolly apple aphid (*Eriosoma lanigerum*), Apple sawfly (*Hoplocampa testudinea*), Green apple aphid (*Aphis pomi*)
Tropical Highlands		Fireblight, Crown rot, Woolly apple aphid
	Strawberry (*Fragaria ×ananassa*)	
Subtropics and temperate zones	Ancient cross of *F. virginiana* (8x) from eastern North America and *F. chiloensis* (8x) from Chile	Grey mould (*Botrytis cinerea*), Powdery mildew (*Sphaerotheca macularis*), Strawberry blossom weevil (*Anthonomus rubi*), European tarnished plant bug (*Lygus rugulipennis*)

Sources: Kranz, 1978; Rehm and Espig, 1984; Mandal, 2006.

in crop evolution (as we have already seen in potato) and has important consequences in breeding clonally propagated crops. It is important to note that all 'breeding lines' or varieties of clonally propagated crops are homogenous (clone lines and varieties are genetically fixed and as homogenous as non-segregating breeding lines or hybrids from breeding self-fertilized or hybrid crops). The homogenous clones are exact genetic copies of their mother plants, if mutations are ignored. This is more or less obvious in potato, cassava or sweet potato field plots, or in fruit and tree plantations, provided no genotype mixtures are observed. What is not directly obvious to an observer is that each clone line or variety in the field or plantation is a highly heterozygous hybrid (clone lines and varieties are highly heterozygous hybrids comparable with heterozygous hybrids developed in hybrid breeding). It should be noted that due to polyploidy, clonally propagated crops are usually more heterozygous than those diploid crops in which hybrid breeding is applied. The difference between "clone hybrids" and "seed hybrids" such as maize is that the first are propagated by asexual reproduction and the latter are developed by sexual reproduction.

13.3 POLYPLOIDY

General knowledge about polyploidy is required to get an understanding of breeding clonally propagated crops. Polyploidy has a strong effect on the performance of clones as well as the parent–offspring correlations. A polyploid genotype contains more than two homologous sets of chromosomes in the nucleus of somatic cells. According to the number of chromosome sets in the nucleus we distinguish different polyploid types: triploid (three sets; 3x), tetraploid (four sets; 4x), pentaploids (five sets; 5x), hexaploids (six sets; 6x) (Tate, Soltis and Soltis, 2005) – and species with even higher polyploidy levels are known (Table 13.1). The haploid level (one set; 1x) does not occur as a normal stage in the life cycle of a crop. However, haploid plants occur by spontaneous mutations, wide crosses and anther culture (e.g. diploids are developed from tetraploid potatoes by pollination with specific clones of *S. phureja* and haploids by anther culture). Haploids are occasionally used in FPB of clonally propagated crops, especially potatoes (Hermsen and Verdenius, 1973; Wenzel and Foroughi-Wehr, 1984). In crop evolution different polyploidy levels originated from genome mutations and by hybridization between very closely related species. Autopolyploids and allopolyploids include wheat (*Triticum durum* and *T. aestivum*), canola (*Brassica napus*) and cotton (*Gossypium* spp.), and nearly all clonally propagated species are autopolyploids. The homologous chromosomes in autopolyploids are similar enough that multivalents of the same homologous chromosomes are formed. Doubling of chromosomes occurs if the spindle poles are not developed when the nucleus is dividing chromosomes in mitotic and meiotic cell division. There are several possible outcomes of abnormal meiosis. Natural formation of 2n gametes was most important in evolution of cultivated *Solanum* species, and the formation is mainly determined by one recessive gene (Watanabe and Peloquin, 1988), so this character can be used in breeding potato. Polysomic inheritance is sensitive to disorders and therefore autopolyploids often have reduced fertility, and occasionally they are completely infertile and propagate only asexually.

Multiple chromosome sets occur spontaneously in nature from 2n gametes and can be induced artificially by colchicine (an alkaloid of autumn crocus, *Colchicum*

autumnale). In the case of diploid plants (2x), this leads to tetraploid plants (4x). An example is the evolution of *S. tuberosum* spp. *andigena* (4x) from cultivated *S. stenotomum* (2x) (Hawkes, 1979). Hybridization of diploid and tetraploid plants forms triploid plants (3x) and by a further doubling of chromosomes hexaploid plants (6x) are formed. An example is hexaploid *I. batatas*, which probably evolved by genome mutation and hybridization, because the sweet potato genome (6x) consists of two closely related sets of chromosomes ($B_1B_1B_2B_2$), of which one is duplicated ($B_1B_1B_2B_2B_2B_2$) (Shiotani and Kawase, 1989; Austin and Huamán, 1996). Many important clonally propagated crops are triploids (3x), such as the economically important genotypes of banana and plantain (*Musa* × *paradisiaca*). The triploid banana and plantain groups evolved in two different ways by genome mutation and hybridization: in the case of banana, from one diploid wild species *M. acuminata* (AA) to form the triploid banana group (AAA), and in the case of plantain, from two diploid wild species: *M. acuminata* (AA) and *A. balbisiana* (BB), forming the triploid plantain group (AAB) (Simmonds, 1962). Many FVs in the banana and plantain group evolved only by somatic mutation, because triploid banana and plantain are infertile. However, breeding triploids is possible by working with two gene pools, one which is diploid and the other which is tetraploid, such as using gene pools of *M. acuminata* and *M. balbisiana* on a diploid and tetraploid polyploidy level to develop new triploid banana and plantain varieties.

In contrast to autopolyploids, the genome in allopolyploids differs so much between hybridized species that only bivalents of homologous chromosomes of the parental genomes can be formed. The breeding behaviour of allopolyploids is very similar to diploids. The formation of bivalents or multivalents appears to be genetically determined, e.g. without the gene (ph) on chromosome 5B in polyploid wheat (*Triticum durum* and *T. aestivum*), homologous chromosomes form multivalents. This gene is relatively new in the evolution of wheat (Dhaliwal, 1977). Among clonally propagated crops, cassava (*Manihot esculenta*) is considered to be a diploidized allotetraploid, which also was formed recently in the evolution of plants (Nassar, 2000). Indications for this are: (i) the high chromosome number (2x = 36) of all *Manihot* spp. (other *Euphorbia* have basic chromosome numbers within the range of six to eleven); (ii) natural hybridization occurs among *Manihot* species and crossing barriers appear to be weak; and (iii) *M. esculenta* shows meiotic irregularities, such as terminal non-pairing, multivalent associations and repetition of chromosome types, which results in low fertility of parental combinations.

Polyploid plants usually have larger plant cells, larger and stronger plant organs, greater height and increased biomass production. In nature, polyploid plants tend to succeed in new habitats. In breeding, the tallest and best thriving plants are selected, so that, unintentionally, many crops have been bred to a higher level of ploidy. However, as chromosome number increases, the increase in biomass production becomes successively less, and production decreases above a specific optimum biomass. This optimum differs from species to species. In autopolyploids, this advantage of increased vigour is associated with the disadvantage of increased meiotic disorders during the formation of multivalents. This is the reason why the harvest in many important autopolyploid

crops is represented by vegetative plant parts. Most polyploids display heterosis relative to their parental species, as well as relative to inter-gene-pool crossings within a species. A polyploid population contains three, four, five, six or more alleles at each locus. Hence, considerably more effects due to dominance and epistasis are possible, and the genetic variation due to dominance and epistatic effects in polyploidy crops is very large compared with the genetic variation caused by dominance and epistatic effects in diploid crops. For this reason, the performance of clonally propagated crops is mainly determined by heterosis. Usually in breeding of clonally propagated crops, an F_1 clone hybrid is crossed with another F_1 clone hybrid, so that the offspring shows extremely extensive segregation. In parent–offspring studies it is possible to determine mid-parent and mid-offspring heterosis, as well as the best-parent mid-offspring heterosis (similar to the assessment of heterosis in a hybrid breeding programme of diploid crops—see Chapter 11). In polyploids, more than one allele per locus is transferred in gametes to the next generation, so that, in contrast to diploids, the genetic variation due to dominance determines the response to selection in population improvement as long as the population is not in equilibrium (after recombining parental material in controlled crossings, a population is usually not in equilibrium). In tetraploid potato populations that are not in equilibrium, one-third of the dominance variance is exploitable for selection progress when selection takes place on the female and male sides (Wricke and Weber, 1986; Gallais, 2004). The exploitation of the dominance variance in population improvement, in combination with the selection for different levels of ploidy (using the inheritance of 2n gametes), has been proposed for breeding tetraploid potatoes (Ortiz, 1998). Polyploidy, heterozygosity and heterosis make the selection of good parents in population improvement of clonally propagated crops very difficult. A good parent generates large genetic variation around a high family mean. Cross-prediction and inter-gene-pool crosses are very important in population improvement of clonally propagated crops. This aspect of clonal breeding is often neglected and this might be the reason for the low level of breeding progress in many clonally propagated crops. In contrast to population improvement (selection of superior parents – see Section 13.7 below), selection within a given genetic variation for variety development is relatively easy in clonally propagated crops (discard inferior material). All the genetic advantages of clonally propagated crops can be used for variety development, and the genotype finally released is in the hands of the breeder immediately after the initial crossings.

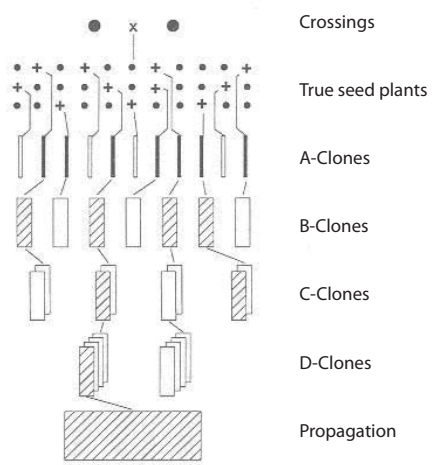
A clonally propagated crop that has no, or nearly no, sexual reproduction is close to a dead end in evolution and breeding. Genetic variability can only accumulate by mutations. However, this source of new variation has often been used to find enhanced types of fruits and ornamentals (van Harten and Broertjes, 1988). Nevertheless, the main source of generating new variation in clonally propagated crops is sexual reproduction. Owing to a more or less regular meiosis in polyploids with an even number of chromosome sets (4x or 6x), sexual seed production and generation of new genotypes is possible. Nearly all clonally propagated crops, e.g. potato, sweet potato and cassava, are cross-fertilized crops in combination with self-incompatibility. Incompatibility alleles are the reason why specifically sought

after cross combinations are difficult to realize, and seeds from controlled crossings can have a very high value in clonally propagated crops.

13.4 GENERAL BREEDING SCHEMES

The general principle of breeding clonally propagated crops is to break normal clonal propagation by introducing a crossing step, which culminates in sexual seed production and genetic variation. After the genetic recombination, all subsequent propagation steps are asexual in nature and done by clonal propagation. Nearly all clonally propagated crops are polyploid and cross-fertilized species. A more or less regular meiosis is possible in polyploids, if the number of chromosome sets is even, as in tetraploids (4x) and hexaploids (6x). The parents in cross combinations are highly heterozygous hybrids, with the exceptions of inbreeding lines generated by self-fertilizations or doubled-haploid and doubled-triploid production. The populations developed from seeds are again formed by very different and highly heterozygous genotypes, which do not exchange genetic material. Each seed plant grown in the so-called seedling nursery can be considered a potentially new variety. This is the basis for selection. The selection between clones is described most often in plant breeding textbooks as a process conducted in several steps (Figure 13.1).

FIGURE 13.1
The general breeding scheme for clonally propagated crops



Source: modified from Becker, 1993.

The breeding scheme illustrated in Figure 13.1 is straightforward and it is most often interpreted as requiring clonally propagated crops to be bred sequentially in several steps over several years. The diagram implies that there are two parents being crossed, followed by five subsequent selection steps in time (one selection step in seed plants and four selection steps in clone plants). This is misleading. First the breeder must work with many parents (further details about number and size of crosses are given in Section 13.5). Second, there is no further genetic development in clonally propagated crops as one moves between selection steps. The selected D-clone in Figure 13.1 is genetically identical to the true seed plant the selected D-clone derives from. Provided that the true seed plant can be cloned in large quantities, it is theoretically possible to test the population with adequate accuracy in the first year to select the 'best' genotype.

Selection among true seed plants is made for tolerance and resistance to pathogens. However, often no selection between plants grown from seed is made by the breeder. Nevertheless, natural selection occurs during germination, and should not be completely avoided, because genotypes difficult to germinate delay the breeding programme. The main reasons for no selection in the seedling nursery are: (i) plants grown from

seed often differ considerably from plants raised from vegetative planting material, (ii) the plants raised from seeds are normally grown in pots in greenhouses, and for most traits this is not representative of field conditions, and (iii) a single plant evaluation is usually not appropriate, with the exceptions of susceptibility to highly aggressive pathogens. In field crops, an important factor is interplant competition. A genotype must be tested under conditions that simulate the field conditions in practice. For this reason several plants of each clone are tested in plots in blocks under homogenous field conditions. The aim is an unbiased comparison of genotypes within blocks. The number of plants per plot and the plot size depends on the crop as well as the breeding stage. Fruit trees and perennial shrubs are tested in larger plots with fewer plants than potatoes, and these again are tested in larger plots than cut flowers. Early selection stages (A-clones and B-clones) are tested in smaller plots than later selection stages (C-clones and D-clones). The amount of planting material at each breeding stage is determined by the propagation coefficient of the crop. For example potato has, among clonally propagated crops, a very low propagation coefficient of about ten, whereas sweet potato has a relatively high propagation coefficient of between 30 and 90 (depending on the field propagation method used). This is one factor why potato breeding is relatively slow (about eight to ten years from cross to variety release).

Breeders do not breed for a single environment; they breed for a range of environments. Hence, the field evaluations must simulate the range of target environments. For this reason, and depending on the propagation coefficient, the clones are tested in plots, in homogenous blocks, at several locations and for several years. It is obvious that the wide range of quality preferences and the numerous pests and diseases in each clonally propagated crop and their interaction with genotypes justifies decentralization and participatory approaches. However, the better simulation of the final target environment realized with FPB justifies a stronger PPB approach. Many advocate PPB because the stress and marginal field conditions of resource-poor farmers are not adequately simulated by FPB (see also below). In this context the two clear advantages of breeding clonally propagated crops should be pointed out: (i) no genetic changes occur in genotypes after seed has been produced; and (ii) the total genetic variation of genotypes (comprising the genetic variances due to additive, dominance and epistatic effects) can be exploited by selection. For these reasons, only the genotype×environment (G×E) interaction and the plot error must be considered (and reduced by testing in several environments) to identify the best clone.

13.4.1 Early breeding stages and PPB

In the general breeding scheme (Figure 13.1) each surviving seed plant is cloned to be raised as A-clones in observation plots (visual screening of general clone performance), or evaluation plots (recording of data on specific traits of each clone). Figure 13.2 shows the planting of sweet potato A-clones. The plot size of A-clones is usually a single-row plot comprising 3 to 5 plants. The trial is conducted with no replications. It is open to discussion whether A-clones should be evaluated at two locations. Selection theory results show that it is nearly always the best resource allocation to test as many clones as possible at one location, without replications (Wricke and Weber, 1986). Many breeders use only one location at the

FIGURE 13.2
Planting early selection stages of sweet potato for the accelerated breeding scheme in San Ramon (one of four locations)

early breeding stages due to the restrictions of the propagation coefficient and breeding budget. However, there are several reasons to test A-clones at two locations: (i) a trial at one location can be lost (e.g. extreme weather conditions) and then a full breeding step and population is lost; (ii) trials at only one location are of little value (the G×E interaction cannot be separated from the genotypic effect); and (iii) the response to selection is still very close to the optimum in a wide range of scenarios, including the scenarios where A-clones are tested at two locations (Grüneberg *et al.*, 2004). Moreover, information from contrasting environments can be combined if the breeder tests A-clones at two locations. For example, clones that clearly fail in a marginal or hot-spot environment (for drought, salinity, biotic challenge, etc.) can be discarded, or at least considered with caution in good environments.

A-clones are only selected for highly heritable traits such as general performance (growth type; tuber, root or fruit size, shape and colour), resistance to pests and diseases, harvest index, dry matter and nutritional quality. Breeders nearly always conduct a visual selection at the A-clone breeding stage. However, it can be questioned if the A-clones selected by the breeder match farmer needs and would be selected by farmers. With two locations, one location can be easily evaluated by farmers in a PPB approach, while the other location is used by the breeder. It should be noted

that visual selection of general performance can also be an efficient indirect selection for yield. In sweet potato, we observed among several thousand A-clones grown in 1 m-row plots at three locations a heritability for yield of about $h^2 \approx 0.4$ (harvesting and recording all A-clones for yield at all three locations). It was considered as 'useless work', because a visual selection at the first location resulted in a nearly common set of selected clones and a heritability for yield of about $h^2 \approx 0$ in the selected fraction. This was demonstrated for two different breeding populations grown in two different seasons, so that the breeding scheme was changed. Only those clones that have passed the visual selection step at location 1 are harvested and considered for storage root quality evaluations at location 2 and 3. However, relying on visual selection in early breeding stages requires a person who is very experienced with sweet potato. We think farmer participation at the visual selection stage in early breeding stages is essential to avoid genotypes entering later breeding stages with characteristics (storage root size, shape, form, colour, etc.) unacceptable to farmers. As described above, farmer preferences vary substantially both within and between regions, and the visual selection can be conducted by independent farmer groups. The advantages of PPB are very obvious in the early selection stages of clonal breeding, in which large numbers of fixed genotypes must be screened for many highly heritable traits. PPB in the early selection stages has been successfully applied in potato (Gabriel and Torrez, 2000), cassava (Manu-Aduening *et al.,* 2006) and sweet potato (Gibson *et al.,* 2008), and by working with two or three locations it can be linked into FPB, in which selection is conducted for traits that cannot be evaluated by farmers, such as nutritional quality (starch, vitamins and micronutrients by fast through-put analysis methods) (Hartmann and Buning-Pfaue, 1998; Lu, Huang and Zhang, 2006; Zum Felde *et al.,* 2007; Bonierbale *et al.*, 2009).

In the next season, B-clones—also called "promising clones"—are planted in larger plots in 2 to 3 rows with planting material obtained from selected A-clones. The B-clone trials are still conducted without replications, but generally at two or more locations. The B-clone stage is usually the beginning of selection for low heritability traits such as yield, biomass and yield stability. The determination of stability parameters such as the slope of the regression line and deviations from regression (Fox, Crossa and Romagosa, 1997) requires at least three locations. However, it should be noted that stability parameters from less than 6 environments are still of little value. As mentioned above, a strong justification for PBB is that stress and marginal field conditions of resource-poor farmers are not adequately simulated by FPB (Ceccarelli, 1994). Cross-over G×E interactions occur, and what appears to be good in resource-rich environments often does not perform well in resource-poor environments. This has also been clearly observed in sweet potato, and outstanding clones for resource-poor environments were discarded by FPB (e.g. the clone SR92.499-23; Grüneberg *et al.,* 2005). Usually, but not always, the response to selection in poor production environments is smaller than in good production environments. The genetic variance is smaller while interaction and error are larger, so that the performance of individual clones becomes more difficult to distinguish. However, outstanding genotypes with different growth types adapted to resource-poor environments cannot display their full potential if FPB

does not test in such environments. Taking sweet potato breeding as an example again, yield stability is associated with harvest index (Grüneberg *et al.,* 2005). Under drought stress, good performing sweet potato clones have a harvest index of about 0.5 (on the basis of fresh matter storage root yields and total fresh matter biomass yields). Vine production is of considerable importance to farmers to obtain sufficient planting material for the next growing season. Clones performing well in resource-rich environments usually fail in drought-stress environments due to insufficient vine production rather than to unacceptable storage root production. At the same time, outstanding clones in drought-stress environments show a strong increase in vine production with medium storage root yields when grown in environments with good water supply (Andrade, unpublished). The selection of genotypes with desired growth types or desired sink–source allocations in marginal environments requires that breeders evaluate the breeding population in such an environment; this characteristic cannot be determined in a resource-rich environment. Here we suggest linking the evaluation in a marginal environment with the visual selection in early breeding stages. All clones that fail in the marginal environment (i.e. extreme reduced storage root production or vine production) are eliminated from all other selection steps.

13.4.2 Later breeding stages and PPB

At the beginning of the C-clone and D-clone selection stages the breeding population has been reduced to between 30 and 300 clones. While the number of clones in later selection stages is further reduced, those selected clones are tested in more environments and in replications. The plots for C-clones and D-clones are 3- to 5-row plots. All important agronomic traits are determined, including taste and post-harvest characteristics. Furthermore, it merits determination of the above-mentioned stability parameters: (i) slope of the regression line, and (ii) deviations from regression, as well as conducting an Additive Main Effect and Multiplicative Interaction (AMMI) Analysis in those cases where the regression model does not fit (Fox, Crossa and Romagosa, 1997). Usually, a clone is considered to have stable performance if the slope of the regression line is close to 1, and the deviations from the regression line are small. An important question in later breeding stages is that of how many locations and how many replications to use. With more locations and more replications, the estimation of the yield performance of clones is more reliable. At the same time, for a given testing capacity, increasing the number of locations and replications results in fewer clones being tested. Generally, the gain from increasing the number of replications is less than that obtained by increasing the number of genotypes and locations. Investigations of this problem in selection theory have led to a recommendation to conduct advanced clone trials still with no replications but in the maximum number of environments that can be managed by the breeder (Utz, 1969, cited in Wricke and Weber, 1986). However, many scientists are still very reluctant to conduct trials without replications. Since the fixed costs of experimental stations are high, it is usually an advantage to (i) create 'artificial environments' on experimental stations (by running part of a station without fertilizer or with less irrigation) and (ii) to go on-farm to evaluate clones with farmers, i.e. PVS. However, nearly all of the initial genetic variation in the breeding population has been discarded at later breeding stages,

so that specific characteristics needed by farmers and consumers are often no longer present in advanced or elite clones if they had not been considered at earlier breeding stages.

13.5 MODIFICATIONS OF THE GENERAL BREEDING SCHEME

The general principle for breeding clonally propagated crops presented above is very simplified. In practice, it is more or less modified. The differences can be large, depending on the crop, country and breeder. For example, resistance or tolerance can already be determined at the true-seed plant stage by eliminating infected plants from the seed nursery. Potato breeders usually try to obtain only a single tuber from each true seed plant to start selection with single plant tests. Clone selection in shrubs and fruit trees uses fewer plants per row and fewer selection stages. Potato breeding uses more selection stages due to the low propagation coefficient of potato. However, there is a common question in all the different breeding schemes: How many genotypes should be selected at each selection stage? In selection of breeding clonally propagated crops this can be easily determined using selection theory. There is an optimum number of clones, locations and replications at each selection step for a given test capacity. Fortunately, the area around the optimum is flat and deviations from the optimum do not have large effects, as long as the deviations are not strong. To select between 5 and 20 percent of the total number of clones at each step is still close to the optimum. However, in the wide range of practical breeding situations, the optimum has always been found in the direction of higher selection intensities, more so than most breeders intuitively realize. It is important: (i) to increase the number of genotypes at the first stage, to the maximum of the available breeding capacity; (ii) to use a high selection intensity; and (iii) to use as many environments as can be managed at each breeding stage (Wricke and Weber, 1986). Replications are of minor importance and should only be used at the final breeding stages. These characteristics of the optimum in multistage selection for clonally propagated crops led to the suggestion of using an accelerated breeding scheme (ABS) for clonally propagated crops in sweet potato breeding (Grüneberg, unpublished).

ABS responds to the frustration that it takes on average seven or eight years from a cross until variety release. Donors are also reluctant to invest in breeding when concrete outputs take so long to materialize. ABS uses the simple fact that in breeding clonally propagated crops each true seed plant is already a potential variety, with the advantages of sweet potato having a very short crop duration (3 to 4 months) and a high propagation coefficient (up to 90 cuttings per plant within 3 to 4 months). ABS overturns the general principal breeding scheme of clonally propagated crops by: (i) crossing and multiplication; (ii) early selection stages; and (iii) late selection stages. Everything that can be implemented simultaneously in these three stages and years is done simultaneously in different environments. However, to reduce labour, every clone that has not met a desired target for a character in the first environment is discarded and not considered (harvested) in the second environment, and the same for characters evaluated in the second environment, and so forth. In selection theory, this multi-trait selection procedure is designated ‘independent culling’ and it is the procedure also used to optimize multistage selection procedures (Cochran,

1951; Wricke and Weber, 1986). In ABS, independent culling is conducted: (i) in a poor resource environment where clones undergo visual selection; (ii) only those clones passing the first selection step are harvested in environments 2 and 3 to determine yield and quality of selected good performance clones over all traits and environments (index values are determined by the Pesek-Baker index (Pesek and Baker, 1969) to assist the breeder in their selection decisions); and (iii) only those clones that have passed the second selection step are harvested in environment 4, where clones were already planted in season 2 in a farmer's field under high SPVD pressure in a third selection step to select for SPVD tolerance. About 300 sweet potato clones enter the later breeding stages. In two subsequent seasons and two selections steps, 4 to 5 clones are finally selected for variety release (first season: 300 clones, three environments, two plot replications and 5-row plots; second season: 40 clones, 16 environments, two plot replications and 5-row plots). This is carried out in cooperation with NARS and farmer groups.

13.6 MAINTAINING VARIETIES AND S-CLONE MULTIPLICATION

As a result of clonal propagation, maintaining varieties should not be difficult. Genetic changes in varieties do not occur by undesired crossings nor by segregation, and mutations are rare. However, the opposite is the truth, and maintaining clonally propagated varieties is a difficult and expensive part of the breeding operation. The main reason is that in clonal propagation through vegetative plant parts, many more diseases can be transmitted compared with seed propagation. A new variety will have no impact in practice, and even can be lost (a clone hybrid developed from two hybrids cannot be reproduced by crossing the hybrids again) without a system that maintains and provides at least some healthy planting material.

Numerous viruses, bacteria and fungi are transmitted by vegetative planting material. Viruses are particularly important, because viral diseases cannot be controlled chemically. Viruses are spread by vectors, most often aphids and whiteflies. The traditional maintenance of varieties and production of healthy planting material includes protecting the base plants of varieties in greenhouses or under nets, and to prevent the development of a vector population by intensive use of insecticides. The base material is also termed 'mother plants'. However, under these conditions, only 20 to 200 plants of each variety can be maintained, and planting material must be produced in the field. These clones in the field for producing healthy planting material are the so-called S-clones, because planting material is usually called seed in clonally propagated crops. Healthy S-clone production is supported by (i) application of insecticides against vector populations (monitoring by yellow cards); (ii) choosing locations for S-clone production that are out of range of vector populations (i.e. locations close to the sea or in cool highlands); and (iii) removing all visibly infected plants from S-clone fields.

The detection of virus infections has been simplified by use of the enzyme-linked immunosorbent assay (ELISA) procedure. The principle is a reaction between the viruses in plants and antibodies against these viruses. The reaction is made visible by an enzymatic colour formation. In practice, some leaf sap is pressed out and the colour reaction is assessed on special test plates coated with antibodies. In the case of sweet potato, the plants tested negative for viruses

are further grafted on an indicator plant such as *Ipomoea setosa* to confirm the absence of viruses for sweet potato viruses. In this way all maintained mother plants of a variety are routinely screened, and only virus-free mother plants are used for further propagation steps. Recently, techniques have been developed to detect viral DNA and RNA directly by real-time polymerase chain reaction (PCR) (Mumford *et al.*, 2006).

However, the best option for maintaining clone genotypes is to start from absolutely virus-free material. This is obtained by *in vitro* propagation of plants under sterile conditions, and these *in vitro* plantlets are the starting point for greenhouse and field propagation. *In vitro* plantlets are replacing mother plants in the greenhouse, often by eliminating all greenhouse plants. If no virus-free material is available, new virus-free plantlets can be obtained by thermotherapy and meristem culture. Meristems of very-fast-growing infected plants are virus free following proper heat treatment, because viruses only start to enter older plant cells. However, this process requires considerable time (at least 18 months for sweet potato, and depends on the virus titre of the infected source plants). In breeding, virus-free material can be achieved by germinating true seed *in vitro* and maintaining these true-seed plantlets *in vitro* until the final selection decision has been made.

Distribution channels for clonally propagated crops are well developed in temperate regions of the world. However, they are almost non-existent in most tropical and subtropical countries, although the pest and disease pressure is considerable higher than in temperate regions. S-clone production in resource-poor environments is nearly all in the hands of farmers, and the health status of planting material is a key factor in high farm yields. Without a certain discipline in S-clone production on farm, the yield level remains low, although virus-tolerant varieties with good overall performance are available. The most important factors for S-clone production on farm are: (i) separating S-clone production from cultivation for production; (ii) removing all visibly infected plants in S-clone field areas; and (iii) obtaining new, healthy planting material at least occasionally from private or public sources. Nevertheless, the private and public seed sectors are an important factor in production of clonally-propagated crops, but this topic belongs to integrated crop and pest management (Salazar, 1996). The breeder's role in this context is to maintain and provide virus-free starter material for the private and public seed sectors.

13.7 SELECTION OF PARENTS AND PREDICTION OF CROSS OUTCOMES

The choice of parents is perhaps the most important step in a breeding programme. Many breeders make several hundred crosses each year and it is often observed that in later steps of the breeding programme the best clones derive from one or only a very few crosses. Hence, there is a desire to predict which cross combinations are most promising. If this were possible, the efficiency of a breeding programme could be increased by reducing the number of cross combinations and increasing the number of genotypes from good cross combinations (produce more genotypes from within the best families). In the situation where not much is known about the performance of a cross, the number of combinations should be increased to the maximum of the breeder's capacity and the number of genotypes per cross should be kept small. The rationale underlying this is based on selection theory, which shows that if "the breeder has no

prior knowledge on the cross … the breeder has to make as many crosses as possible", which is also minimizing the risk of raising genotypes with poor performance (Wricke and Weber, 1986). As mentioned above, most clonally propagated crops are polyploid and highly heterozygous, so that dominance and epistatic effects contribute considerably to clone performance. For this reason, it should be assumed that not much is known about the value of a cross combination until it has been made and tested. This is in agreement with our observations in sweet potato, where the correlation between mid-parent and mid-offspring yields is low ($r \approx 0.5$). We currently recommend raising 10 to 20 genotypes per cross combination, while increasing the number of cross combinations to the maximum possible with the resources available. However, after clones of these crosses have been evaluated, the good crosses should be repeated on a large scale. An optimum for the number and size of crosses can determined if estimations are available for the genotypic variance between crosses and within crosses, and the non-genetic variance components (Wricke and Weber, 1986). Breeders often generate a large number of seeds in polycross nurseries, but in these only the female parent is controlled. The correlation between parent and mid-offspring in breeding populations derived from polycross nurseries is half of mid-parent–mid-offspring correlation in controlled crosses.

Often the parents are chosen due to their performance *per se*. For theoretical reasons, this cannot be very secure in clonally-propagated crops. Clone varieties are highly heterozygous hybrids and usually polyploids, so that segregation in crossings is almost unpredictable. Therefore, for a long time now, suggestions have been made for better assessment of parents; however, they are rarely used in practice. One suggestion is to determine the value of a parent on the basis of the offspring performance from test crosses. Another suggestion is to work on a reduced polyploidy level, which has been especially proposed for breeding tetraploid potatoes (Ross, 1986). However, the latter has been little applied in practice for parental selection, but has often been used to incorporate germplasm of wild *Solanum* species into advanced breeding populations (Tarn *et al.,* 1992). Parental selection on the basis of test crosses are made on a large scale in potato breeding programmes for long-day, temperate climates (150 to 500 cross combinations per breeding programme, cited by Ross, 1985). It has been observed that specific combining ability is nearly as large as general combining ability, and in some cases specific combining ability has been observed that is clearly larger than general combining ability (Sanford, 1960; Mullin and Lauer, 1966; Tai, 1976; Killick, 1977; Veilleux and Lauer, 1981; Gaur, Gopal and Rana, 1983, cited by Tarn *et al.,* 1992; Gopal, 1998; Kumar, 2004; De Galarreta *et al.,* 2006). This is not surprising as long as potato breeders do not work with two clearly separate gene pools for variety development. In potato breeding for tropical and subtropical regions, heterosis and high general combining ability have been observed between *andigena* and *tuberosus* gene pools in tuber-propagated potatoes and in true-seed potatoes (Enrique Chujoy, pers. comm.). However, as long as these gene pools are not improved on the basis of general combining ability separately from the complementary gene pool, such effects cannot be exploited in the long term.

In sweet potato experiments we observed a mid-parent–mid-offspring heterosis of 84 percent among 48 cross combinations (or 184 percent if the mid-parent value is set to

100 percent). This is a clear indication that the design of breeding schemes using the combining ability of two gene pools merits investigation. Two breeding gene pools are available for sweet potato to test heterosis: the Jewel Gene pool, developed mainly from North American varieties, and the Zapallo-SPK Gene pool, developed mainly from South American and African FVs.

The value of a parent is nearly always determined by several characteristics. In general, parents should be recombined with a good combining ability and good performance over all traits. The PPB study in Uganda (Gibson *et al.,* 2008) underlines how many characteristics are important for good performance over all traits. Moreover, FPB also has the aim of improving nutritional quality, especially pro-vitamin A, iron and zinc concentrations (Pfeiffer and McClafferty, 2006) in potato, sweet potato, cassava, plantain and other crops. With an increasing number of characters, breeders operate with larger breeding populations, as in potato and sweet potato. Aiming at only 30 genotypes finally selected, and assuming 10 characters each, selected in sequential selection steps with a selected fraction of ten percent (1 out of 10), then 300 000 000 000 genotypes would be needed in the original base population. Populations of this size cannot be established in practice. Moreover, even if the population size is extremely large, some desired combinations probably do not exist, such as sweet potato genotypes with high yield, high SPVD tolerance, high DM and high pro-vitamin A, iron and zinc concentrations). Often, breeding can only approach the desired genotype in several steps of recombination and selection. In practice, some characters are selected sequentially (especially where there is clearly a lowest acceptable value (tuber size, shape and colour, as well as pest and disease resistances), while others are selected simultaneously by aggregating characters into an index (often an intuitively formed index, such as score values for overall performance).

A parent appears to have a good overall trait performance if no trait is below the population average. However, only in those cases where trait associations are close to zero or positive can it be expected that parents with good performance over all traits produce offspring in which each character has been improved. In parental selections, negative trait associations can be very critical. Table 13.3 gives an example for sweet potato, in which DM shows a strong negative trait association with pro-vitamin A, iron and zinc concentrations, as well as a moderate negative trait association with storage root yield. The associations in the example are strong enough that under various scenarios of multi-trait selection the breeding population is improved for yield, pro-vitamin A, iron and zinc, whereas the DM of the population decreases.

In other words, the DM is changing in the wrong direction even though it was selected for improvement. These surprising undesired effects in the case of sweet potato and DM improvement in connection with pro-vitamin A, iron and zinc improvement was also observed for the Williams selection procedure and this index selection procedure (Williams, 1962) comes very close to intuitive selection procedures used by breeders in which a weight is assigned to each trait on the basis of its economic importance. The only selection procedure that can monitor the response to selection in each trait is the Pesek Baker index (Pesek and Baker, 1969). However, this index requires estimations of genetic variance and co-variances, but the procedure ensures that parents are selected that

TABLE 13.3
Estimations of genetic correlations for yield, dry matter, total carotenoids, iron and zinc in sweet potato storage roots of 24 megaclones and 26 advanced breeding clones grown in at two locations in two replications

	Storage root yield	Dry matter	Total carotenoids	Iron
Megaclones (orange and white fleshed)				
Dry matter	-0.49			
Total carotenoids	-0.06	-0.54		
Iron	-0.26	-0.23	0.94	
Zinc	-0.22	-0.39	0.93	0.74
Advanced breeding clones (only orange fleshed)				
Dry matter	-0.54			
Total carotenoids	0.55	-0.71		
Iron	-0.24	-0.07	0.14	
Zinc	0.39	-0.20	0.37	0.53

develop populations in which traits are improved according to a ratio of desired genetic improvements (so-called desired genetic gains) given by the breeder.

An alternative is the Elston index (Elston, 1963), in which the breeder can raise the threshold for the trait at risk by modifying the lowest acceptable value for each. This index can be easily applied in each replication and environment, so that index mean values for each genotype can be calculated together with other statistical parameters (Grüneberg *et al.*, 2005).

We are aware of only one case in which PPB has been applied for the selection of parents in clonally propagated crops. In the Cochabamba region of Bolivia, farmers selected potato parents in an *andigena* population, which had been improved for agronomic performance and Late blight tolerance. Selected clones in this population were used as parents with the regionally grown FV 'Waycha' (Gabriel and Torrez, 2000) and the PPB approach included hand-crossing by farmers. We think that the ability of farmers in the selection of parents is limited beyond a selection of clone performance *per se*. Test crosses, general combining ability, specific combining ability and improving gene pools on the basis of general combining ability values (called reciprocal recurrent selection in maize breeding) are the most difficult tasks in breeding; however, they can greatly increase yield gains. At the same time, we think that the visual selection of potential parents in a PPB approach should be used as additional information by the breeder. It should be noted that the work plan for both the selection of parents for the next cycle of selection and the early selection stages for variety development are always to a certain extent in common. In sweet potato breeding at CIP we use a combination of sequential and simultaneous index selection in early selection stages (see also above): (i) visual selection by eliminating all genotypes that do not meet the lowest acceptable values for each trait (this lends itself to PPB); (ii) in the remaining selected fraction (about 2 500 clones), apply index selection for yield and nutritional quality traits using the Pesek-Baker index, with the square roots of variance components as desired genetic gains; and (iii) selecting for pest and disease tolerance (mainly SPVD) in the remaining selected fraction (about 300 clones) by visual selection (this lends itself to PPB) and

ELISA. The remaining 100 to 200 clones enter later breeding stages, but are also used as parental material for the next cycle of recombination and selection. In such a breeding system, with one population, two PPB steps can easily be applied. However, a PPB approach is feasible also in inter-pool crosses linked with general combining ability improvement. Farmers select in families (derived from recombining the two gene pools) in early generations for variety development (as described above). The interesting information for the breeder provided by farmers could be the numbers of selected clones per family. With this information the breeder can focus only on those parents in the improvement of the separate gene pools, which for the farmer results in interesting cross combinations with the other gene pool. On top of this, the breeder can use the opportunity to apply the general combining ability concept. This would be a very elegant PPB approach for selection of parents and cross prediction. Although Hull (1945), in his fundamental paper on reciprocal recurrent selection, proposed this for breeding clonally propagated crops, this method of clonal breeding is rarely found in practice.

The topic has been considered in breeding clonally propagated trees (e.g. Baudouin *et al*., 1997; Kopp *et al*., 2001; Pâques, 2004) and recently discussed by Miles (2007) in the frame of apomixis for cultivar development in tropical forage grasses. The proposed "evolutionary breeding approach" for *Musa* spp. (Ortiz, 1997) is also in the narrow sense a reciprocal recurrent selection scheme. However, subsequent application of reciprocal recurrent selection is rarely found in practice, although we think that this is the way ahead to exploit heterosis and achieve more breeding progress in clonally propagated crops.

13.8 APOMIXIS

As mentioned earlier, the principle advantage of breeding clonally propagated crops is that each clone variety is fixed and maintainable. However, this is associated with the disadvantage of vegetative propagation. Diseases are easily transmitted and the maintenance of varieties and the production of healthy planting material are expensive. The ideal propagation system for clone varieties would be vegetative propagation by seeds. This ideal propagation system exists in nature, and is called apomixis (Nogler, 1984). Apomixis is the formation of seeds without meiosis, and two forms are distinguished: (i) agamogenesis (also called gametophytic apomixis), in which the asexual embryo is formed from an unfertilized egg; and (ii) adventitious embryony, in which the asexual embryo is formed from nucellus tissue. Apomictically produced seeds are genetically identical with the parent plant. The breeding work on apomictic species is very difficult and requires developing population improvement by sexual reproduction and subsequent variety development by apomixis. Apart from some forage (Miles, 2007) and citrus species (Soost and Roose, 1996), apomixis is not used in plant breeding.

The difficulty in breeding apomictic crops is the development of genetic variation. However, in populations with a high frequency of apomictic plants, both facultative apomicts and completely sexual plants can usually be found, and such genotypes can be used to develop new genetic variation. For breeding, it is important to find or develop a system in which both apomixis is maintained (variety development) and sexual reproduction is restored (population improvement) so as to be able to develop new genetic variation. This can be compared to male sterility

systems used in hybrid seed production. It is interesting that apomixis is distributed across many plant families. It appears not to be controlled by a complex genetic system. An example is Guinea grass (*Panicum maximum*), in which the sexual tetraploids are recessive homozygous (aaaa), whereas apomictic genotypes carry a dominant allele and are heterozygous (Aaaa) (Savidan, 1983). So far, studies on apomixis have been mainly made in tropical grasses, but more and more attention is being paid to rice and maize. There are opinions that apomixis systems will become available to breeders, and in this context gene isolation and an 'apomixis gene' have been mentioned (Savidan, 2000). However, so far there is no such apomixis system usable in breeding programmes. The major problem is that plants with the same genotype can express different degrees of apomixis.

13.9 PROPAGATION OF POTATOES BY SEED

Finally, the option that clonally propagated crops can be propagated by sexual seeds is considered. In many countries there have been research projects in which potatoes were cultivated by seed. These are potatoes that are sown instead of planted. Since the planting material of clonally propagated potatoes is often called a 'seed' potato, the term 'true potato seed' (TPS) was introduced. The use of TPS has two principle advantages: the most important potato diseases cannot be transmitted in true seed, and only a few hundred grams of TPS are needed to cultivate a potato field, where usually several tonne of tubers are needed (Simmonds, 1997). This is associated with two disadvantages: potatoes grown from seed are weak in vigour and are sensitive to many factors, and the breeding method and advantages of breeding clonally propagated crops can no longer apply. Moreover, breeding TPS potato as a cross-fertilized crop will not lead to completely homogenous varieties. This is the reason why only a few TPS varieties have been developed in the Northern Hemisphere. All these have been exclusively used for home garden production. However, we think that by using hybrid selection schemes and inbreeding in two separate gene pools it should be possible to develop more and more homogenous and attractive TPS varieties.

The advantages of TPS are mainly of interest in tropical and subtropical regions of the world. Under these climatic conditions, the production, storage and transportation of potato planting material is difficult. Moreover, potato yields are considerable lower in tropical and subtropical regions of the world than in the Northern Hemisphere, so that about 20 percent of the harvest is needed as planting material. Hence TPS varieties in the tropics can have 20 percent lower yields compared to clonally propagated potato varieties and remain competitive. About 20 TPS varieties have been developed. Most interesting are those varieties developed from recombination of the *andigena* and *tuberosus* gene pools. However, the original idea of raising seedlings in nurseries and then planting seedlings into the field by hand has not been adopted. What has been adopted is to raise TPS varieties in seedling nurseries to obtain healthy planting material, and then to cultivate these TPS varieties for several growing seasons as a clonally propagated crop, and to request true seed again after yield declines are significant due to declining health status (Fuglie, 2001). However, today, not more than 10 000 ha of TPS are grown, mainly in Asia, which trace back to about eight TPS varieties. The future of TPS is debatable. From the breeding perspective,

the future of TPS will mainly depend on working with two gene pools, in which a certain extent of inbreeding is applied, with subsequent use of general combining ability to improve these two gene pools.

13.10 POTATO

Breeding potatoes has been reviewed by Tarn *et al.* (1992). The andigena potato (*Solanum tuberosum* subsp. *andigena*; autotetraploid with 48 chromosomes) originated in the highlands of South America about 5000 BC, while today two-thirds of world potato production is in temperate latitudes. Following introduction into Europe, andigena evolved into the Irish potato (*S. tuberosum* subsp. *tuberosum*), which is mainly characterized by day-length neutrality, uniformity of tuber shape, shorter crop duration and higher harvest index than andigena. Andigena remains the predominant cultivated potato in the Andes, whereas the Irish potato is the potato of commerce in long-day temperate climates. Potatoes introduced into other, tropical, regions of the world trace back to breeding populations derived from crossings between andigena and Irish potato. However, in the Andean region, seven other potato species are still in cultivation; most important are phureja (*S. phureja*; diploid with 24 chromosomes), limeña, ajanhuri and rucki. In addition to these cultivated species, 160 wild potato species are known (Hawkes, 1979 and 1981; Spooner and Hijmans, 2001), so that potato might have the largest gene pool among crops. Wild and indigenous species are important resources of pest and disease resistance for andigena and Irish potato. The evolution of the potato was described in the introduction of this chapter. Asia and Europe are the world's largest potato producing regions, with annual production of about 130 and 128 million tonne, respectively, followed by the Americas (41 million tonne) and Africa (16 million tonne) (FAO, 2006). The top 20 potato producing countries are China (22 percent), The Russian Federation (12 percent), India (8 percent), Ukraine (6 percent), United States of America (6 percent), Poland (3 percent), Germany (3 percent), Belarus (3 percent), Canada (2 percent), France (2 percent), United Kingdom (2 percent), Turkey (2 percent), Netherlands (1 percent), Bangladesh (1 percent), Brazil (1 percent), Romania (1 percent), Peru (0.8 percent), Spain (0.6 percent), Nepal (0.5 percent) and Pakistan (0.5 percent).

Breeding objectives

Characteristic of potato breeding is the large number of breeding objectives. For the Irish potato, quality traits are at least as important as yield. Moreover, breeding for resistance against numerous pest and diseases, e.g. numerous viruses (potato leaf-roll virus (PLRV), Potato virus Y (PVY) and Potato virus X (PVX)), Late blight (*Phytophthora infestans*), dry rots (*Fusarium* spp.), soft rot and blackleg (*Erwinia* spp.), cyst-forming nematodes (*Globodera rostochiensis* and *G. pallida*) have major importance in long-day temperate as well as in tropical temperate climates. For the andigena potato, these pests and diseases are of nearly similar importance (i.e. late blight can destroy the whole crop in cool, high-altitude regions, especially when the weather is wet).

There are clear differences between tropical temperate and tropical hot climates. At temperatures above 25°C, Late blight and cyst-forming nematodes decline rapidly in importance, but Early blight (*Alternaria solani*) and root-knot nematodes (*Meloidogyne* spp.) take over, and Bacterial wilt (*Pseudomonas solanacearum*) is widespread in tropical

lowlands. The phureja potato (for PVY and Late blight) and the wild species *S. acaule* (for PLRV, PVX and both *Globodera* spp.) and *S. demissum* (for PLRV, PVY, and late blight) are important resources in breeding for tolerance and resistance. It should be noted that the pests and diseases presented represent only the most important species. Ross (1985) provides a list of resistance sources in wild potato species.

However, it is possible to find tolerance or resistance genes in cultivated and wild potatoes against nearly all potato diseases. An exception is Bacterial wilt, for which so far no useful tolerance or resistance have been found for breeding purposes. Today, all new Irish potato varieties contain one or more resistance genes from wild and other cultivated potato species. For the Northern Hemisphere, yield, crop duration, tuber size, shape and flesh colour, eye depth, starch content, storability, cooking characteristics, taste and suitability for mechanical harvesting, as well as processing characteristic for chips (crisps) and French fries (chips) are the most important quality breeding objectives. For tropical regions, yield, regional adaptability, crop duration, storability, cooking characteristics, taste and nutritional quality are the most important quality breeding objectives. Outside of the Andes, crop duration is one of the most important traits (i.e. in south-west and central Asia there is a requirement for potato varieties with less than 80 days crop duration). Recently, focus has been given to improve pro-vitamin A, iron and zinc concentrations in tubers to alleviate micronutrient malnutrition in tropical regions (potatoes have comparatively high iron and zinc concentrations). This has resulted in a separate breeding programme for phureja, which has the highest iron and zinc contents among potatoes, together with considerable levels of total carotenoids, including pro-vitamin A.

Breeding methods

Crossing is relatively easy. In nature, crossings occur easily by open pollination by insects. For breeding purposes the flower architecture of the potato allows easy emasculation and controlled hand pollination. A fruit with about 200 seeds develops from each successful pollination. In commercial breeding, controlled crossings are usually made (both parental genotypes are clearly defined). Genotypes with good performance over all traits and with a certain degree of genetic distance are recombined. The value of a cross combination is usually determined in test-crosses with 100 to 200 seeds per combination. Occasionally plant breeding text-books recommend combining parents with complementary traits. However, many breeders find that this results in potatoes breeding in a 'wild' segregation, so that finally only genotypes can be selected with moderate performance over all traits. Each year, breeders plant 10 000 to 200 000 seeds, which trace back to 150 to 200 cross combinations. Crossings are made with flower sprouts obtained from cuttings of field plants, grown in greenhouses in nutrient solutions. Frequencies of successful crosses differ tremendously between parental combinations and about one-eighth to one-quarter of all parental combinations cannot be recombined due to no flowering, low pollen quality, no fruit formation or genetic incompatibility. For an overview of overcoming crossing barriers in potatoes, the reader is referred to Jansky (2006).

Pre-breeding crosses are important when one requires to improve one or two traits in an enhanced breeding gene pool (e.g. shorter crop duration). Pre-breeding

is usually made in two or three cycles of recombination and selection in which the desired traits are incorporated in a genetic background that is more close to the enhanced breeding gene pool. This is generally done for resistances genes from a wild parent or exotic variety. It often involves an additional selection step at a different polypoidy level. The re-synthesis of tetraploids by mitotic duplication of diploid genotypes (colchicine treatment of seed, axillary buds, tuber germs, leave explants or callus) is usually not recommended, because mitotic tetraploids have considerably lower yields than meiotic tetraploids. Meiotic tetraploids occur naturally in crossings between tetraploid and diploid potatoes (4x × 2x) due to meiotic anomalies that result in unreduced gametes (Rowe, 1967; Jacobsen, 1980).

Breeding potato for tropical regions of the world aims mainly at improvement of four gene pools: (i) the andigena (A) gene pool for short-day high altitudes; (ii) the andigena × tuberosus (AT) gene pool for short- and long-day temperate regions, with emphasis on selection for Late blight tolerance and PVY and PLRV resistance; (iii) the tuberosus × tuberosus (TT) gene pool for short- and long-day warm regions, with an emphasis on selection for short crop duration; and the (iv) phureja gene pool (P), with emphasis on nutritional quality. In the A, AT and TT gene pools at CIP about 60 parents are recombined by controlled crossings to raise about 20 000 seedlings, whereas in the P gene pool the number of recombined parents is considerable lower (about 30 parents). The selections start in seedling populations for both resistance and tuber formation. In three selection steps the material is reduced to about 300 clones, which are evaluated in 2-row plots with two replications. Later selection stages include evaluation at several locations in replicated plots. However, the propagation coefficient in potato is very low (≈ 10) so that it takes about eight to ten years before final selections enter the variety release and dissemination stage.

Today there is little investment in TPS in the original sense. However, selection of families that are to a certain degree homogenous in crop duration, tuber form and shape, with some genetic diversity in tuber yield and specific adaptation, are of interest. The reason is that these can be disseminated as seeds and farmers have the option to exploit genetic variation for specific adaptation in a PPB approach. PPB has been successfully applied in Bolivia in early breeding stages (Gabriel and Torrez, 2000) and in later breeding stages (FVS) in Ecuador (Bonierbale, pers. comm.).

13.11 SWEET POTATO

Breeding sweet potatoes has been reviewed by Martin and Jones (1986), Laurie and van den Berg (2002) and Grüneberg *et al.* (2009). The sweet potato (*Ipomoea batatas*, Convolvulaceae, hexaploid with 90 chromosomes) is also known as *batata, camote* or yam (United States of America). The crop was domesticated in tropical America about 6000 BC and reached the Pacific and south-east Asian islands naturally or by early seafarers before Columbus. The number of wild species in the genus *Ipomoea* is large (more than 500 species). However, no wild form of *I. batatas* has been found. It is assumed that *I. batatas* developed from an interspecific cross between a diploid and a tetraploid *Ipomoea* species in the *I. trifida* complex. It is possible to re-synthesize new *Ipomoea* hexaploids by hybridization of diploid *I. leucantha* and tetraploid *I. littoralis* (Nishiyami, Miyazaki and Sakamoto, 1975.). The Spanish introduced sweet potato in the

sixteenth century into the Philippines, whence it spread to other islands and the east Asian mainland. Portuguese seafarers introduced the crop into Europe, Africa and India. Today it is cultivated in 117 countries in all tropical and subtropical regions of the world. Asia is the world's largest sweet potato producing region, with about 107 million tonne of annual production, followed by Africa and the Americas, with approximately 15 and 3 million tonne, respectively. The top 12 producing countries are China (80 percent), Nigeria (2.8 percent), Uganda (2.2 percent), Indonesia (1.5 percent), Viet Nam (1.2 percent), United Republic of Tanzania (0.9 percent), Japan (0.8 percent), India (0.8 percent), Burundi (0.7 percent), Kenya (0.6 percent), Rwanda (0.6 percent) and United States of America (0.6 percent). Further important sweet potato producing countries are Angola, Argentina, Bangladesh, Brazil, Cuba, Egypt, Ethiopia, Haiti, Korea (Democratic Republic of), Madagascar, Peru, the Philippines and Papua New Guinea, with annual production between 0.3 and 0.5 million tonne (FAO, 2006). Nearly half of the sweet potato produced in Asia is used for animal feed, with the remainder primarily used for human consumption, either as fresh or processed products. In Africa, the crop is cultivated almost exclusively for fresh consumption.

Sweet potato is a perennial vine, propagated by cuttings, and usually cultivated as an annual crop. The planting distances in fields vary. In Africa, planting distances are usually 1 m between rows and 30 cm within rows. In China, recommended planting distances are 75 cm between rows and 20 cm within rows. The crop duration is very short (4 to 6 months) and the crop is even cultivated in northern China. It produces more edible energy per hectare per day than wheat, rice or cassava, and is well adapted to salinity, drought and marginal soil conditions (Woolfe, 1992).

The crop has recently received more interest due to the very high levels of pro-vitamin A (concentrations of up to 700 ppm DM) in OFSPs, and hence as a vehicle to reduce vitamin A deficiency problems in the world (Huang, Tanudjaja and Lum, 1999; Low, 2007). We observed up to 1 200 ppm β-carotene on a DM basis in clones with variety potential in our breeding population 'Jewel II' (this corresponds to 30 mg β-carotene in 100 g fresh sweet potato storage roots. A pre-schooler needs 4.8 mg β-carotene per day, and it merits discussion as to what extent OFSP should be recommended as baby and weaning food. Moreover, storage roots provide medium levels of iron and zinc (Woolfe, 1992). Recent finding of about 50 ppm DM iron and 40 ppm DM zinc in deep orange fleshed sweet potato storage roots (Burgos and zum Felde, pers. comm.) merits further investigation.

The stems and leaves can have spinach-like taste and some varieties are used in China specifically as a green vegetable. Stems and leaves have on DM basis about four times more protein, iron and zinc than storage roots. It appears that stems and leaves must be cooked to reach an acceptable iron bioavailability, but investigations into iron bioavailability of sweet potato tops is very limited.

There is new demand for purple-fleshed sweet potato due to the health-promoting effects of anti-oxidant anthocyanin substances, and cell lines for a potentially ongoing production for the food industry have been established (Konczak, 2006). However, much more important appears to be the demand for non-sweet sweet potatoes, but few genotypes are non-sweet (Kays, 2006). There is a very large genetic

variation for DM, starch and sugars in sweet potato, and a strong positive correlation has been observed for DM and starch, whereas a strong negative correlation was found between sugars and DM and starch (Grüneberg *et al*., 2009). This is nearly ideal for the breeding target of a non-sweet high-DM sweet potato type, and we think that the development of non-sweet sweet potatoes should not be too difficult.

Breeding objectives

FPB started very late for sweet potato. One of the first breeding programmes was established at Louisiana State University in the 1920s. Today there are several strong national breeding programmes (e.g. China, Japan, South Africa, Uganda, United States of America and Uruguay) and one international breeding programme, at CIP (Peru). Four major breeding objectives can be clustered: (i) breeding of OFSP for consumption of storage roots and leaves; (ii) breeding for high DM and extractable starch; (iii) breeding for biofuel production, which has started in China (Dai Fu Ma, pers. comm.); and (iv) breeding of purple-fleshed sweet potatoes for consumption. In breeding for consumption, it has to be considered that people in different regions have very different taste preferences; the extremes are low DM content, moist mouth feel, very sweet taste and deep orange flesh colour, versus high DM, bland, dry mouth feel, low sweet taste and white, yellow or orange flesh colour. In breeding for human consumption, focus is more on high DM OFSP varieties with elevated iron and zinc concentration and a dry and less-sweet mouth taste. This breeding is hampered by a strong negative genetic correlation between storage root DM and storage root pro-vitamin A, iron and zinc contents. The breeding for human consumption includes the use of the crop as animal feed and folder. The breeding for high DM and extractable starch is relatively easy: the target is a high starch yield per hectare. However, currently, in many regions of the world the price of sweet potato starch currently cannot compete with the price of cassava starch. Only in large regions where the growing period is too short for cassava within the cropping system (e.g. China) is there an economic demand for sweet potato varieties for starch production. Breeding for biofuel production is in its initial stages, and so far variety recommendations for this purpose are made on the basis of screening existing successful varieties. The breeding of purple-fleshed sweet potatoes as a separate breeding programme is a relatively new trend, and so far only carried out on a small scale in Japan, Indonesia and Peru. Future targets are the non-sweet sweet potato, and quick cooking features (cv. Quick Sweet) (Katayama *et al.*, 2006), as well as suitability for processing into chips, puree, juice, weaning and baby food, and bread on the basis of a wheat-sweet potato flour mixture (Woolfe, 1992); these trends appear nearly exclusively in east Asia, and for recent developments the reader should consult proceedings, such as Liu (2008).

Major constraints on high yields are pests and diseases, especially Sweet potato chlorotic stunt virus (SPCSV) and the sweet potato weevils. The prevailing diseases and insects affecting sweet potato vary from region to region. There are about 35 bacterial and fungal diseases, more than 20 viruses or virus-like agents, 20 nematodes and 20 insect species known to affect sweet potato (Martin and Jones, 1986).

Currently there are only four important pest and diseases: SPVD, Alternaria, sweet potato weevils and the root-knot nematode. The most important virus is whitefly-

transmitted SPCSV, which often occurs in co-infection with Sweet potato feathery mottle virus (SPFMV – aphid-transmitted). Clear synergistic disease effects are seen with SPFMV and SPCSV (the so-called SPVD virus complex). Generally, all varieties need a certain degree of tolerance to SPVD, and there is genetic variation for SPVD (Mwanga, Yencho and Moyer, 2002). Very high tolerance or resistance is needed in eastern Africa. Currently, it is assumed that SPFMV and all other sweet potato viruses (except SPCSV) are not important, because sweet potato has an effective virus defence system, which is broken by SPCSV (I. Barker, pers. comm.).

The major fungal disease in subtropical America is Fusarium wilt (*Fusarium oxysporum* f.sp. *batatas*) and in the African highlands the main problem is Alternaria storage root, leaf spot and stem blight (*Alternaria* spp.). Although there are many bacterial and fungal diseases with a wide distribution, high levels of tolerance or resistance are frequently found. This is also true for resistance to nematodes.

There has been recurrent success in breeding for root-knot resistance against new races of *Meloidogyne* spp. (Martin and Jones, 1986). However, in regions with a pronounced dry season, the greatest constraints are sweet potato weevils (*Cylas formicarius elegantulus* in all parts of the tropics, *C. puncticollis* and *C. brunneus* in Africa, and *Euscepes postfasciatus* in the West Indies). It has been an objective to find weevil resistance for more than 50 years, but differences in weevil attack probably depends on preference factors of the weevil. It is believed that dense storage roots developed deep below the soil surface are less susceptible than less dense, moist-fleshed storage roots. No effective weevil resistance has been found so far. For this reason, a transgenic approach using *Bt* genes has received attention. Recent findings of compounds in the latex of the storage root skin and the effect of these on weevils might of interest for breeding (P.C. Stevenson, H. Muyinza, D. Hall and R. Mwanga, unpubl.).

Breeding methods

True seed set occurs easily in nature by cross pollination (by insects, mainly bees), and for breeding purposes the flower architecture of sweet potato allows easy emasculation and controlled hand pollination. A skilled technician can make 200 crossings per day, with a success rate of 25 percent. From each successful cross, two or three true seeds are obtained. Not all sweet potato parents flower readily, but flowering can be easily induced by grafting on *Ipomoea nil* (2n = 30 chromosomes). It should be noted that frequencies of successful crosses differ tremendously between parental combinations, and about one-third of all parental combinations are incompatible, with no seed formation. The sweet potato seed has a hard coat and needs to be scarified with concentrated sulphuric acid to obtain even and rapid germination. In a well managed breeding nursery, after 3 months it is possible to obtain 40 to 60 cuttings from a true seed plant if the plant is grown in the field, and 20 to 30 cuttings if the plant is grown in a pot in a greenhouse. The extreme genetic make up of the crop (hexaploid, highly heterozygous, open-pollinated by insects with true seed set occurring easily), the short crop duration (4–5 months), and the rapid propagation (40 to 60 cuttings from one plant) permits the design of a very efficient and rapid breeding system.

The recombination of parents is still usually carried out in polycross nurseries

by open pollination. Polycrosses have been considered as very efficient in sweet potato breeding (Martin and Jones, 1986). However, theoretically controlled crosses must be more efficient, provided that high selection intensities can be reached, which depends on technical skills and costs. Only a few breeding programmes are making (at least to any major extent) controlled crosses (e.g. in China, Mozambique, Peru and Uganda). The numbers of recombined parents vary between 20 and 120, and the number of genotypes raised per population (true-seed plants) varies between 5 000 and 30 000. Selection of parents is almost exclusively carried out on the parental performance *per se*. In China, Uganda and at CIP in Peru, the information from progeny test crosses is used to repeat good cross combinations on a larger scale (2 000 to 3 000 genotypes per cross). In recent years, CIP has established two genetically divergent populations to test heterosis and general combining ability in applied breeding material. There are plans to change from a selection of parents by parental performance *per se* to a reciprocal recurrent selection scheme based on general combining ability. Selection of genotypes for variety development is usually carried out as described in the section of the general breeding scheme for clonally-propagated crops. Starting with recombining parents, it takes on average 7 to 8 years until variety release. At CIP, Peru, an accelerated breeding scheme is used in which temporal variation of test environments are replaced by spatial variation of test environments. This accelerated breeding scheme takes on average 3 to 4 years until variety release. It appears that there are funding opportunities to implement this breeding scheme in Africa, particularly in Ghana, Uganda and Mozambique.

13.12 CASSAVA

Breeding cassava has been reviewed by Byrne (1984), Bonierbale *et al.* (1994) and Ceballos *et al.* (2004). Cassava (*Manihot esculenta*, Euphorbiacaea, diploid with 36 chromosomes) originated in South America. The crop is also known as *manioc* and *yucca*. Wild *Manihot* species—weedy sub-shrubs, shrubs and trees—are principally found in dry regions of Mesoamerica and South America. The highest density of diversity is found in west-central Brazil. Many wild *Manihot* species show considerable tuber production and it is assumed that *M. esculenta* was selected from one or several of these wild species in the northern part of South America or in west-central Brazil. The crop was disseminated by tribal migrations and its variability increased by selection for agronomically preferred types and further hybridization with wild species. Cassava was introduced in the fifteenth century into West Africa by the Portuguese from Brazil, and from there it spread to eastern Africa, Madagascar and southern India. Moreover, it was introduced in the sixteenth century into the Philippines by Spanish traders from Mesoamerica. Today the crop is cultivated worldwide in lowland tropics. World production of cassava root was estimated to be about 226 million tonnes in 2006, with most production in Africa, where 122 million tonnes were grown, while 67 million tonnes were grown in Asia and 37 million tonnes in Latin America and the Caribbean (FAO, 2006). The top ten cassava producing countries are: Nigeria (18 percent of world production), Brazil (12 percent), Thailand (10 percent), Indonesia (9 percent), Democratic Republic of the Congo (8 percent), Ghana (5 percent), United Republic of Tanzania (4 percent), India (4 percent), Mozambique (3 percent), and Angola (3 percent).

Cassava adapts to a wide range of ecological conditions and is known for its tolerance of low soil fertility, drought and pests. The growing period is long, between 7 and 18 months. The yields are very high (about 30 to 40 t/ha under commercial practice). However, the protein content of cassava is low (<3 percent DM), which makes the crop ideal for starch production. Cassava is often grown in low input production systems, particularly when it is grown as a food crop. Planting material is easily obtained from plant stems available from the farmers' own or neighbouring fields. About 70 percent of cassava is grown by small-scale producers for direct human consumption. The crop tolerates more drought, lower soil levels of nitrogen, potassium and phosphorus, lower pH and higher aluminium levels than most other crops. Under these conditions, yields are about 7–10 t/ha. Cassava is often found in mixed stands, together with a variety of other food or cash crops. Estimates indicate that at least one-third of the cassava grown worldwide is intercropped (Cock, 1985).

Breeding objectives

FPB started in isolated programmes in the early 1900s when cultivation was extended by several colonial governments as a safeguard against famine, and breeding new clones with resistance against cassava mosaic disease (CMD) was required. Cassava breeding programmes started in Brazil (in the 1930s), India (in the 1940s), Indonesia (in the 1950s) and at two international institutions: CIAT, Colombia, (in the 1970s) and IITA, Nigeria, (in the 1970s). These institutions have developed a very successful cassava breeding network.

In cassava breeding, three diseases have been the highest priority for decades: (i) Cassava mosaic disease (CMD), which is a whitefly-transmitted virus widespread in Africa and India; (ii) Cassava brown streak disease (CBSD); and (iii) Cassava bacterial blight (CBB), caused by *Xanthomonas campestris* pv. *manihotis*, which can have devastating effects on yield in Africa. Of regional importance in Latin America and the Caribbean is Frogskin disease, suspected to be caused by a virus. Aside from these, cassava is much less affected by disease than other tropical crops, the only other two of importance being Cassava anthracnose disease (*Colletotrichum manihotis*) and root rots (*Phytophthora drechsleri* and *Rhizoctonia* spp.) (CIAT 2001; Hillocks and Wydra, 2002).

The major pests of cassava are nematodes (*Meloidogyne* spp.), whiteflies as a vector of CMD, Cassava green mites (*Mononychellus* spp. and *Tetranychus* spp.), cassava mealybug (*Phenacoccus* spp.), and the grasshopper (*Zonocerus elegans*). Pests and diseases, together with poor cultural practices, combine to cause yield losses as high as 50 percent. In the late 1980s, a new strain of CMD occurred in Uganda that made the virus more harmful. This mutated virus has been spreading and is now found throughout Uganda, Burundi, Cameroon, the Democratic Republic of Congo and Rwanda (Thresh and Cooter, 2005). Next in importance in breeding are more short and thick storage roots with high starch content. This is important for mechanical harvesting, but makes also manual harvesting easier. It is desirable for the roots to be as far as possible horizontal in the soil and near to the soil surface. Breeding selects for plants with lower height and higher harvest index. In cassava breeding for human consumption, the focus is on yield and quality such as low fibre, low levels of cyanogenic glucosides, high protein, elevated pro-vitamin A, iron and zinc concentration in the storage roots, reduced

post-harvest physiological deterioration and regional preferences for the peel of the roots (Ceballos *et al.*, 2004). Cassava varieties are often categorized as either 'sweet' (actually 'not bitter') or 'bitter', signifying the absence or presence of toxic levels of cyanogenic glucosides. The so-called 'sweet' cultivars can produce as little as 20 mg/kg cyanide in fresh roots, while 'bitter' ones may produce more than 50 times as much. Additionally, an important breeding objective is to develop more clones with high adaptation to drought-prone environments. The genetic variation in cassava for pro-vitamin A concentrations is small. However, breeding for yellow cassava genotypes with a pro-vitamin A concentration of 15 ppm appears to be possible. Additionally, a transgenic approach is used to introduce the β-carotene pathway into cassava (J. Tohme, pers. comm.). Breeding for commercial production also selects for plants with shorter height and higher harvest index – giving more stability and resistance against storms – and extensive branch formation, quickly forming a full canopy of leaves not too close to the soil (Byrne, 1984).

Breeding methods

Cassava is a monoecious, highly heterozygous plant. All 36 chromosomes show regular bivalent pairing at meiosis. However, in both cassava and *Manihot glaziovii* (sect. Arboreae) there is evidence of polyploidy from studies of pachytene karyology. There are three nucleolar chromosomes, which is high for true diploids, and duplication for some of the chromosomes. This indicates that *Manihot* species are probably segmental allotetraploids derived from crossing between two taxa whose haploid complements had six chromosomes in common but differed in the other three (Nasser, 2000). Cassava shows self-fertility with strong inbreeding depression and wide segregation in cross progenies. Time of flowering depends on the genotype. There are types in which flowering starts about two months after planting, as well as types that do not flower until after 24 months or more. This makes planned recombination difficult. Earlier and more abundant flowering is obtained by foliar application of indole acetic acid (IAA) and naphthalene acetic acid (NAA). The female flowers are large, nearly always located at the base of the inflorescence, and open first. The female flowers normally open 10–14 days before the males on the same branch, but self-fertilization can occur because male and female flowers on different plants of the same genotype can open simultaneously.

The proportions of self- and cross-pollinated seed produced depends on genotype, planting design and the type of pollinating insects present (5 percent self-pollination occurs naturally). Both the stigma and the pollen are sticky and pollination is easily carried out by honey bees. In the Northern Hemisphere, cassava usually flowers from July to January, with a peak between September and November. In the Southern Hemisphere, it usually flowers from January to July, with a peak between March and May. Tall plants with less branching are less floriferous than highly branched, low growing ones. To make a controlled cross between two parents, unopened flowers are first enclosed in muslin bags and the chosen pollen applied to the stigmas as soon as the female flowers open. The muslin bags are then replaced with netting bags to catch the seed when the ripe fruits dehisce explosively. The fertility of clones is variable and can be very low; an average of one or two seeds per fruit is common in controlled pollination. Seed matures 70 to 90 days after pollination. The fruits are collected when the coat begins to

shrivel and are sun dried until they shatter, releasing hybrid seeds that are ready for germination. Cassava seed have a very short dormant period and germinate quickly. No scarification is necessary. Few seeds germinate unless the mean temperature exceeds 24°C, with a temperature exceeding 30°C for at least part of the day; the best rates occur at 30–35°C. A dry heat treatment of 14 days at 60°C is also beneficial for newly harvested seeds. If temperatures permit and irrigation is available, the easiest method is to sow the seeds direct into the soil. This is successful at IITA because temperatures from January to March range from 30° to 35°C. At CIAT, seeds are frequently planted in a screen house and the emerging seedlings held until they reach 20–25 cm before being transplanted to well prepared soil with good moisture conditions.

Since many national programmes do not have a continuous cassava crossing programme, they rely on distribution of pre-selected clones from the two international institutions, CIAT and IITA. The improved germplasm generated is distributed either in the form of elite genotypes transferred *in vitro*, or as populations of recombinant seeds (full-sibs or half-sibs). Cassava breeding operates with larger populations than potato or sweet potato.

In West Africa, up to 100 000 true seed plants are raised from field-sown seed, which are screened in a first selection step for resistances to CMD and CBB. At harvest, selection is for compact roots with short necks, stems branching at about 100 cm, with low HCN in the leaves. In the second selection step, about 3 000 clones are grown in small, non-replicated plots. Further selection is made for disease resistances, yield potential and root DM content, and the HCN in the roots is assayed enzymatically. For the third selection step, ca. 100 clones are tested in replicated trials at three locations, and consumer acceptance is assessed. Final selections are multiplied and enter dissemination in year 6.

In eastern and southern Africa, 10 000 to 50 000 true-seed plants are raised for the first selection step and screened for resistance to major diseases and pests at 1, 3, 6 and 9 months after sowing, namely East African cassava mosaic disease (EACMD), African cassava mosaic disease (ACMD), Cassava brown streak disease (CBSD) and CBB. In a second selection step, 2 000 clones are planted in single-row plots (3 to 5 plants) at 1×1 m spacing. The observations made in the first year are repeated again at 1, 3, 6 and 9 months after planting. Each clone is scored for yield, and agronomic characteristics assessed, such as branching height and angles, canopy and number of stems per plant. In a third selection step, 20 to 50 clones are grown in preliminary yield trials in single rows with ten plants per clone and three replications at one to three locations. In year 6, the final selections are taken on-farm and into national variety release trails.

In the Americas and Asia, cassava improvement is closely linked with the institutions Embrapa (Brazil), FCRI Rayoung (Thailand) and CIAT (Colombia). In contrast with Africa, there are no extremely devastating diseases. CIAT established 50 000 seedling selections for particular climatic zones. Up to 20 parents from each gene pool are disseminated for evaluations to national centres in similar edapho-climatic zones. From this programme a very broad range of improved diversity has been developed and distributed worldwide.

Generally, MVs in Asia can be traced back to 100 crosses between Asian and American parents (Kawano, 2003). Recent findings show that the general combining ability for cassava fresh root yields are clearly larger

than the specific combining ability across contrasting environments (Ceballos *et al.*, 2004). This is a clear indication that heterotic gene pools in cassava can be formed and exploited by improving two gene pools with a reciprocal recurrent selection scheme.

13.13 BANANA OR PLANTAIN

Breeding bananas and plantains has been reviewed by Rowe (1984), and Jain and Swennen (2001) have edited recent proceedings on banana improvement, with a main emphasis on biotechnology. Banana and plantain (*Musa* × *paradisiaca,* Musaceae, usually triploid with 33 chromosomes) originated in Southeast Asia. The term plantain is used for those bananas that are palatable only when cooked. The crop was introduced into Africa about 3 000 BPE. Introduction into the Americas came after 1 500 AD. Today the crop is cultivated worldwide in the tropics. Bananas and plantains evolved from two diploid wild species, *Musa acuminata* (AA) and *M. balbisiana* (BB) in the Eumusa series (x = 11) of the genus *Musa*. An exception is the small group of 'Fehi ' bananas in the Pacific, which have their origin in the Australimus series (x = 10) of *Musa*. All export fruit bananas are triploids (AAA) and originated from *M. acuminata*. All plantains and several locally preferred fruit bananas are hybrids between *M. acuminata* (AA) and *M. balbisiana* (BB). The higher dry matter (about 5–8 percent) and higher starch content of plantains compared to pure *M. acuminata* cultivars is attributed to the BB genome. The AAB cultivars have long curved fruits and appear like an oversized export banana. They are important food crops in south India, eastern and central Africa and tropical America. The ABB cultivars have thick straight fruits, which are much shorter than the AAB types (Simmonds, 1976; Ortiz, 1995). They are a staple in Samoa, the Philippines, south India and the West Indies. Around 87 percent of all bananas and plantains grown worldwide are produced by small-scale farmers for home consumption or for sale in local markets. About two-thirds of world production is dessert bananas and one-third plantains. The fruit export market comprises only one-sixth of total world production. The banana is the number one fruit crop in the world, with about 70.5 million tonne produced annually. The top ten producing countries are India (24 percent), Ecuador (9 percent), Brazil (9 percent), The Philippines (8 percent), China (8 percent), Indonesia (5 percent), Costa Rica (3 percent), Mexico (2 percent), Thailand (2 percent) and Colombia (2 percent). Plantains are grown as a staple food in 52 countries worldwide with a total production of 34 million tonne. The top ten plantain producing countries are Uganda (30 percent), Colombia (9 percent), Rwanda (8 percent), Ghana (7 percent), Nigeria (6 percent), Peru (5 percent), Cote d'Ivoire (4 percent), Congo (4 percent) and Kenya (3 percent) (FAO, 2006).

Bananas and plantains are one of the very few crops in which breeders are still trying to find an appropriate conventional breeding method to develop new MVs. Nearly all cultivars are FVs and have been selected from genetic variation developed by natural evolution. In cases of crop failure due to new pathogens and diseases, FPB still focuses on identifying alternative cultivars within existing genetic variation (collections and large screening programmes). Hence, an important source for identifying 'new' cultivars are germplasm collections held in trust in genebanks, such as the International Musa Germplasm Collection in Leuven, Belgium. Spontaneous mutants in *Musa* have played a very important role in banana and

plantain breeding, including the replacement of the export banana cultivar 'Gros Michel' (susceptible to Panama disease or Fusarium wilt (*Fusarium oxysporum* sp. *cubense*) by 'Cavendish' banana cultivars, which are resistant to most fusarium wilt pathogens, and the replacement of the plantain cultivar 'Horn plantain' (AAB) (susceptible to Black sigatoka (*Mycosphaerella fijiensis)*) by the 'Laknau' cultivar (AAB), which is tolerant to Black sigatoka and closely resembles the Horn plantain (Stover, 1972). However, the cooking qualities of Laknau are inferior to Horn plantain. Owing to the low level of occurrence of spontaneous mutations, mutagenic agents and mutation breeding have often been used to generate new genetic variation in bananas and plantains, followed by screening programmes for plants with resistance or tolerance to pest and diseases, coupled with desirable agronomic qualities (e.g. tolerance to Panama disease; tolerance to the toxin of *Mycosphaerella fijiensis*; short; larger fruit size; and earliness). The FPB programmes for bananas and plantains started in the early 1900s, to develop new AAA cultivars for the export market, with resistance against Panama disease or Fusarium wilt (*Fusarium oxysporum* f.sp. *cubense*). Despite continued breeding efforts, no new banana and plantain cultivar acceptable by farmers and consumers was bred until the 1980s (Roux, 2001). Nevertheless, by the end of the twentieth century, efforts to improve *Musa* started to focus on the use of diploid and tetraploid gene pools to develop triploid and tetraploid bananas and plantains. To date, the first improved cultivars (AAA, AAAA, AAB, AAAB and AABB), developed at *Fundación Hondureña de Investigación Agrícola* (FHIA) in Honduras through the International Musa Testing Program (IMTP), have been widely distributed. However, for several of these FHIA cultivars, taste and cooking qualities are still problematic (Roux, pers. comm.). Further breeding programmes have been set up at the *Empresa Brasiliera de Pesquisa Agropecuaria* (Embrapa) in Brazil, the *Instituto de Investigaciones en Viandas Tropicales* (INIVIT) in Cuba, the *Centre Africain de Recherches sur Bananiers et Plantains* (CARBAP) in Cameroon, the International Institute of Tropical Agriculture (IITA) in Nigeria, and the National Research Centre on Banana (NRCB) in India.

Breeding objectives

In breeding, resistance against Panama (Fusarium wilt) and sigatoka diseases are in the foreground. In the first half of the twentieth century, Panama disease destroyed approximately 40 000 ha of bananas in Central and South America. Fortunately, resistant Cavendish cultivars could substitute for the predominantly grown Gros Michel variety. However, Cavendish cultivars are not resistant to all fusarium wilt pathogens (i.e. race 4). It should be noted that Panama disease cannot be controlled chemically, so that use of resistant varieties is the only way to maintain production in regions with challenge from this disease. The leaf spot diseases caused by *Mycosphaerella musicola* (Yellow sigatoka) and *M. fijiensis* (Black sigatoka) are costly pathogens and must be regularly controlled by fungicides. Cultivars with an AAA genome are very susceptible to both sigatoka diseases. The Horn plantain is resistant to Yellow sigatoka, but susceptible to Black sigatoka. The latter disease threatens continued cultivation of the plantain food crop. Triploid cooking bananas of the ABB type, such as 'Chato', 'Pelipita' and 'Saba', are highly tolerant to the Black sigatoka pathogen. However,

Chato is susceptible to bacterial wilt or Moko disease caused by *Pseudomonas solanacearum* and to race 2 of *Fusarium oxysporum* f. sp. *cubense*, while Pelipita does not meet flavour and fruit-shape preferences, so that currently only Saba remains as a possible substitute for the Horn plantain. Moreover, nematodes, mainly the burrowing nematode (*Radopholus similis*), are a major constraint to bananas in monoculture, and outside of the Americas the Bunchy top virus is widely distributed, which is transmitted by the banana aphid (*Pentalonia nigronervosa*). Many diploid accessions of *M. acuminata* subsp. *malaccensis* and *M. a.* subsp. *burmannica* are resistant to races 1, 2 and 4 of Panama disease. Sources of resistance to Yellow sigatoka are available in several subspecies of *M. acuminata,* while *M. a.* subsp. *burmannica* is highly tolerant to the Black sigatoka fungus. The tolerance in *M. acuminata* accessions to sigatoka diseases is apparently controlled by several dominant genes. Resistance to the burrowing nematode has been found in the 'Pisang Jari Buaya' group of diploid accessions. The resistance is controlled by one or very few dominant genes and has been incorporated into diploid and polyploid progenies. Today, several FHIA varieties are resistant to burrowing nematodes (Kalorizou, Gowen and Wheeler, 2007).

Among agronomic qualities, dwarfness is most important in bananas and plantains, because they are often grown in areas with periodic strong winds. Dwarf and semi-dwarf mutants have been found in many diploid and triploid bananas and plantains. Examples are 'Highgate' (a dwarf mutant of Gros Michel) and the Cavendish cultivar 'Grand Nain'. In dwarf diploids, the dwarfness character is controlled by a single dominant gene. After this in importance are fruit characteristics and tillering capacity (Ferwerda and Wit, 1969; Rowe and Richardson, 1975; Persley and De Langhe, 1987).

Breeding methods

Triploid bananas and plantains are vegetatively parthenocarpic, i.e. no pollination is necessary for fruit development. In diploids, pollination often results in seeded fruits. Diploids are not suitable as varieties since fruit size and plant vigour are low. However, diploids are the basis for crop improvement. In the initial stages of breeding efforts, a few seeds per bunch in some triploid varieties were used when these had been pollinated by diploid genotypes. The reason for this seed production and genetic variation is the formation of unreduced triploid gametes in some triploid female parents after pollination within diploid male parents, which produces reduced haploid gametes. The progenies of these crosses are tetraploid. This method was used to generate genetic variation with the female banana parent Gros Michel and the female plantain parent Laknau (AAB), which closely resembles the Horn plantain. Tetraploid hybrids (AAAA) from crosses with Gros Michel were resistant to Panama disease and closely approached commercial acceptability, but the inferior agronomic characteristics of the diploid parents were also present in the hybrids. Triploid hybrids derived from crosses between these tetraploid hybrids and diploid genotypes were useless. Unfortunately, the cooking qualities of hybrids derived from Laknau were also inferior to those of the Horn plantain. No seeds have been produced from Cavendish clones and no other suitable triploid parents—except Gros Michel and Laknau—for seed production by unreduced gametes have been found. This breeding method has not succeeded in creating acceptable new varieties. However,

the major finding of this work was that it is necessary to improve the diploid male parent gene pool to increase the chances of developing either new tetraploid or new triploid varieties.

Today, banana and plantain breeding aims at producing tetraploid and triploid varieties on the basis of diploid accessions resistant to various diseases, and the continuous improvement of this diploid gene pool for agronomic qualities (i.e. plant height, fruit characteristics and tillering capacity) as well as high pollen production. Crossings within the diploid gene pool are complex: the diploid 'SH-2095', which was later successfully used in tetraploid variety development, was derived from a four-way cross of three diploid cultivars and one wild accession (('Sinwobogi' × 'Tjau Lagada') × ('Guyod' × a wild *Musa acuminata* subsp. *malaccensis*)). Nevertheless, the genetic basis of diploid pollen parents with improved agronomic performance is considerably wider than in the past. The currently best diploids are continually crossed on triploid Highgate and Laknau, which produces unreduced triploid gametes, as well as on seed-fertile tetraploids with good agronomic performance. The first results in new potentially tetraploid varieties and the later in new potentially triploid varieties. The advantage of triploids in variety development is that they are female-sterile due to the uneven number of chromosome sets. In contrast, the even number of the chromosome set in tetraploids requires an additional selection step for female sterility in variety development. Several improved FHIA cultivars (AAA, AAAA, AAB, AAAB and AABB) have been developed by this breeding method, and farmers participate in the final breeding stages in acceptability studies of these tetraploid and triploid varieties (i.e. PVS) (Ssemwanga, Thompson and Aked, 2000; Ludger, 2005; Kalorizou, Gowen and Wheeler, 2007). However, to our knowledge, no PPB has been applied in early breeding stages. Most likely the reason for this is that almost no diploid clone in its performance *per se* would achieve acceptability by farmers. Nevertheless, the future of banana and plantain breeding, as in other clonally propagated crops, should be seen in testing the combining ability between two gene pools and in the improvement of two gene pools on the basis of the general combining ability and reciprocal recurrent selection. In banana and plantain breeding, such a breeding system can be established by a seed-fertile diploid gene pool with high pollen production and a seed-fertile tetraploid gene pool, which is used as the male parent. In such a breeding programme, PPB could easily be incorporated. However, the important information provided by the farmers would not be seen in the evaluation of clone performance *per se* in the diploid and tetraploid gene pool, but in the numbers of acceptable clones per cross combination and family between genotypes of the diploid and tetraploid gene pool, as described above in the section on selection of parents and cross prediction.

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CHAPTER 14

Breeding for quantitative variables. Part 1: Farmers' and scientists' knowledge and practice in variety choice and plant selection

Daniela Soleri and David A. Cleveland

14.1 INTRODUCTION

This chapter focuses on the knowledge and goals for selection of Third World farmers in comparison with those of formal plant breeders. By Third World farmers (hereafter simply 'farmers') we mean those in the relatively marginal (high stress, high spatial and temporal variability) growing environments of small-scale, traditionally-based agricultural systems (hereafter simply 'small-scale' or 'Third World' agriculture).

The assumption of conventional economic development has for decades been that these farmers would soon be absorbed into the industrial sector, and food production would shift to large-scale, industrial farms, and this scenario is still seen as desirable by many (e.g. Conway, 2003). There is, however, evidence that this small-scale Third World (SSTW) agriculture remains necessary for feeding a significant proportion of the world population, and will probably be necessary in the future, even with production increases in large-scale, industrial agriculture (Hazell *et al.*, 2007). More than 2 billion people live on almost 500 million small-scale farms (<2 ha) in the Third World, including half of the world's undernourished people and the majority of people living in absolute poverty (Nagayets, 2005). Economic re-structuring beginning in the 1980s removed government support for SSTW agriculture and led to migration from rural to urban areas, creating a crisis there (Hazell *et al.*, 2007; Narayanan and Gulati, 2002; Wise, 2007). In addition to irreplaceable food production, SSTW agriculture has other benefits: it operates in many of the world's centres of crop genetic diversity, where farmers conserve diversity in the form of crop genetic resources *in situ*, along with rich cultural and linguistic traditions (FAO, 1996; Harlan, 1992). Plant or crop genetic resources comprise wild and weedy relatives of crops in addition to farmers' varieties (FVs), which include landraces, traditional (folk) varieties selected by farmers, modern varieties (MVs) adapted to farmers' environments by farmer and natural selection, and progeny from crosses between landraces and MVs (sometimes referred to as creolized or degenerated MVs) (Berg, 2009; Cleveland, Soleri and Smith, 1994; FAO, 1996; Zeven, 1998). Sustaining and increasing crop production is essential for the survival of SSTW agriculture, and, in this, seed saving and plant breeding have critical roles to play

In this chapter we review theory and data on selection by farmers, and compare it with selection by formal, scientific plant breeders (hereafter simply 'plant breeders' or 'breeders'). Because selection by farmers and formal plant breeders is based on the same basic biological principles, their understanding and practice of selection may be similar. However, there are differences between farmers and breeders in the genotypes and environments they work with, including the types of agricultural systems for which they are selecting, as well as differences in their experiences, technologies and goals for selection. Similarities and differences in selection among farmers and among formal plant breeders also exist, for the same reasons. Our goal in this chapter is to review what we know about these similarities and differences, and why understanding them is important for collaboration between farmers and breeders to improve selection for varieties that could help SSTW farmers survive and prosper in the future.

We believe that respect for farmers and their knowledge is essential for achieving the maximum benefits from collaborative plant breeding. The greatest single mistake plant breeders and other outside scientists can make is to assume they understand local agricultural systems. Even if their hypothe-

ses are accepted through local research, new details and perspectives are sure to arise, and it is only by having an open minded, respectful attitude that outsiders can hope to learn and reap the benefits of collaboration with local farmers. Such an attitude facilitates new insights and understandings that can improve the accuracy and relevancy of the scientific work. If plant breeders think of their interactions with farmers as tests of how complete or accurate farmers' knowledge is, breeders will lose a critical opportunity for supporting collaboration, respect and collegiality, and for improving the quality of their own work. Thus, at least initially, experiments and discussions with farmers should be seen as opportunities to learn, not to teach.

14.1.1 Choice as distinct from selection

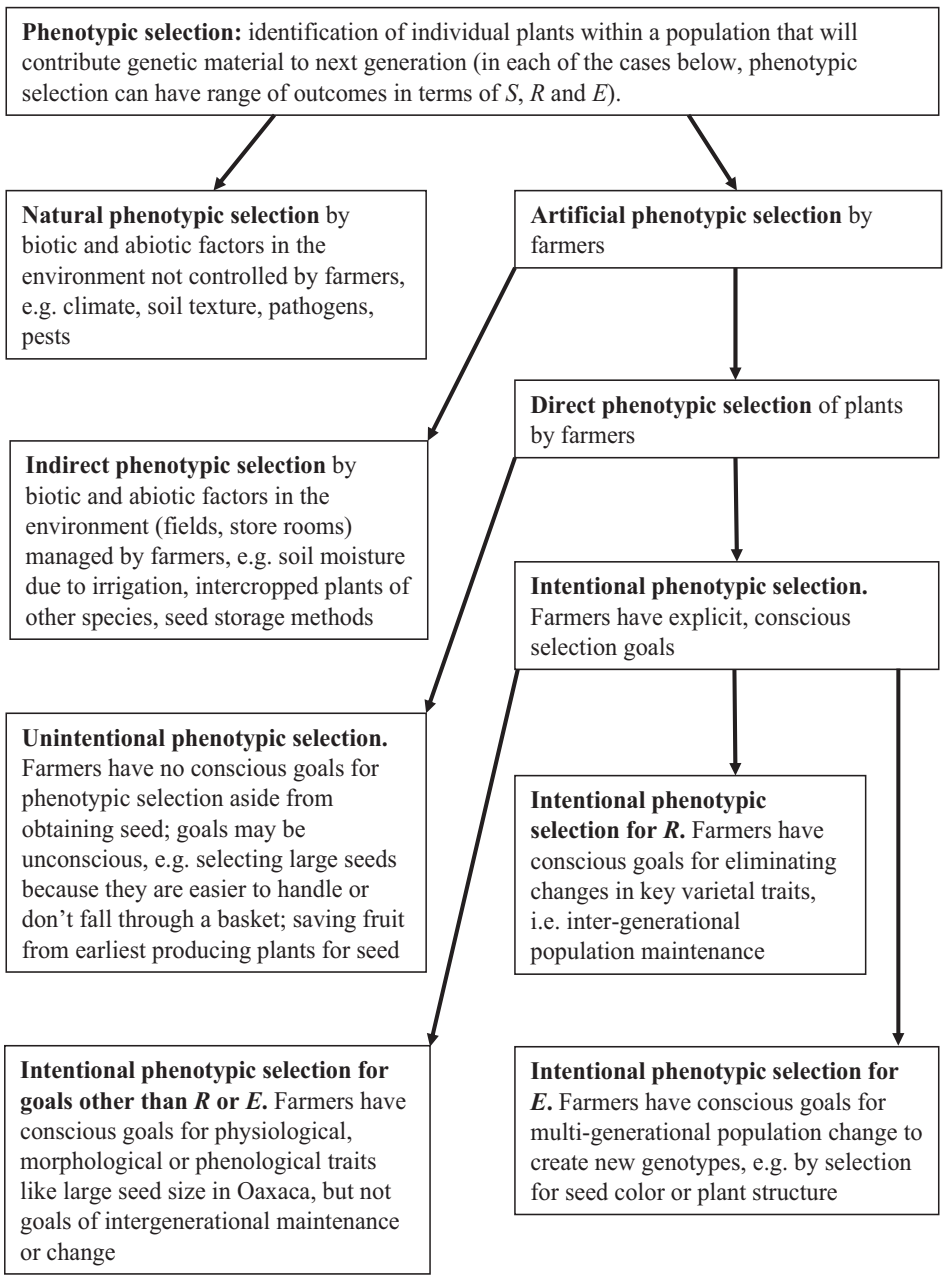
It is important to differentiate between *choice* of populations or varieties that does not change the genetic make-up of these units, and the *selection* of plants from within populations or varieties, with the potential to change the genetic make-up of these units, which may eventually result in new varieties (Cleveland, Soleri and Smith, 2000). Farmer criteria for both choice and selection include agronomic, economic, culinary and aesthetic characteristics, as well as minimizing perceived risk. While the distinction is commonly made in some participatory plant breeding literature (e.g. Witcombe *et al.*, 1996), the terms 'choice' and 'selection' are often not explicitly defined, and in some writing may be used interchangeably. Obviously, distinguishing between these is partly a function of scale, as is most clearly seen in the case of vegetatively propagated crops, in which a single clone may be chosen to establish a new variety (e.g. with cassava; Pujol, David and McKey, 2005). It also depends on definitions: a farmer's variety of a self-pollinated crop (e.g. of barley or rice) may be composed of diverse genotypes that, from a plant breeder's perspective, may be different varieties. Therefore discriminating among these genotypes would be selection from the perspective of farmers as it can change the genetic make up of their variety, but would be choice from the perspective of plant breeders as it would not change the genetic make-up of varieties as they define them. At a more fundamental level, farmers' choice of populations and varieties determines the diversity available for hybridization and subsequent selection of plants. For all of these reasons, we can say that selection and choice together determine the degree to which varieties stay the same, change between generations, or evolve over generations.

Farmers and plant breeders make choices between varieties and populations, especially in the initial stages of the selection process when choosing germplasm for making crosses (for plant breeders), and in the final stages when choosing among populations or varieties generated from those crosses for further testing (Hallauer and Miranda, 1988: 159), or for planting (farmers) or release (plant breeders). Farmers' choices of varieties or populations when saving seed for planting, in seed procurement and in allocating different varieties to different growing environments also affects the genetic diversity of their crop repertoires, and establishes the diversity on which future selection will be based. (For simplicity, in the discussion of choice we will use the term 'variety' to refer to both populations and varieties.)

14.1.2 A taxonomy of farmer selection

A taxonomy of selection and its biological effects can help to clarify the differences

FIGURE 14.1
Phenotypic selection classified according to the agent of selection, and farmers' goals when the farmers are the agents, applied to traditional agricultural systems



See text for key to symbols.

and similarities between plant breeders and farmers. Selection can be categorized according to the agent carrying out phenotypic selection, and the intention of the agent when it is a human (Figure 14.1). While all types of selection function in both farmer and professional breeding, professional plant breeders see intentional phenotypic selection for micro-evolution over generations (*E*) as the primary goal, with other types of selection either eliminated (e.g. applying irrigation to eliminate drought selection), controlled for (e.g. in experimental plot design to reduce σ_E^2), or used to optimize selection for *E* (e.g. roguing off-types) (Cleveland and Soleri, 2007).

Figure 14.1 focuses on selection under farmer conditions. *Natural selection* is not influenced by farmers, in contrast with human or *artificial selection*. Artificial selection is both *indirect*, a result of the environments created by farmers and plant breeders, e.g. in their fields and store rooms, and *direct*, a result of human selection of planting material. Direct artificial selection can be both unconscious or *unintentional* (based on implicit or correlated criteria), when no conscious decision is made about the trait selected for, and conscious or *intentional* (based on explicit criteria), the result of decisions to select for certain traits.

14.1.3 A biological model to compare farmer and plant breeder knowledge and practice

Many plant breeders and other outsiders who work with farmers make the mistake of assuming that western scientific knowledge and practice is always more accurate and 'better' than that of farmers. To have a way of comparing plant breeder knowledge (PBK) and farmer knowledge (FK), a neutral comparator that can function as a bridge between these is useful (Soleri and Cleveland, 2005). For plant breeding, the most fundamental model of the relationship among phenotype, genotype and environment is assumed to be a good model of reality that is the basis for PBK; we will assume it is also the basis for FK. This model is universally accepted by biologists, including plant breeders, but they disagree among themselves about its interpretation at higher levels of generalization, for example whether selection in optimal or marginal environments leads to genotypes that are better adapted to marginal environments (Ceccarelli and Grando, 2002) (see Chapter 2). This variation in scientists' interpretations suggests that, if farmers do in fact think in terms of this basic biological model, it would be a valuable comparator, facilitating understanding of variations (differences in higher levels of its interpretation) within and between FK and PBK on equal grounds.

We use the two parts of the model on which plant breeding is based (Cleveland, Soleri, and Smith, 2000), as presented in standard texts (e.g. Falconer and Mackay, 1996: 189; Simmonds and Smartt, 1999: 193).

1. Variation in population phenotype (observable characteristics) (σ_P^2) on which choice (discrimination between different groups of plants) and selection (discrimination among individual plants within a group) are based is determined by genetic variation (σ_G^2), environmental variation (σ_E^2), and variation in genotype (genetic constitution)-by-environment (G×E) interaction (σ_{GE}^2), thus $\sigma_P^2 = \sigma_G^2 + \sigma_E^2 + \sigma_{GE}^2$.
2. Response to selection (*R*) for a trait is the difference between the mean of the whole population from which the parents were selected and the mean in the next

generation produced by planting those selected seeds under the same conditions. R is the product of two factors, h^2 and S ($R = h^2S$), where S is the selection differential, the difference between the mean of the selected parental group and the mean of the entire original population (Allard, 1999: 101–102; Falconer and Mackay, 1996: 189; Simmonds and Smartt, 1999: 193). Narrow sense heritability (h^2) (that part of σ_P^2 that can be passed directly from parent to progeny, the additive variance, σ_A^2) = σ_A^2/σ_P^2. Thus, artificial phenotypic selection *per se* is a process of identifying the individuals with specific phenotypic traits within a population that will contribute genetic material to the next generation, and is distinct from the heritability of those phenotypic traits (see Section 14.5).

In our use of the basic biological model, we make several assumptions. (1) It models empirically observable patterns in the real world. (2) Among both farmers and plant breeders and other scientists, there are some who are particularly good observers of their environments, crops and interactions between these if they occur, while others are poor observers, resulting in variation within groups. (3) Variation in knowledge within and between groups can also be caused by experiences with different genotypes and environments, and by different values and pre-existing knowledge. (4) Differences between FK or PBK and the model do not mean that either form of knowledge is wrong, and differences between FK and PBK do not mean that either is inferior to the other.

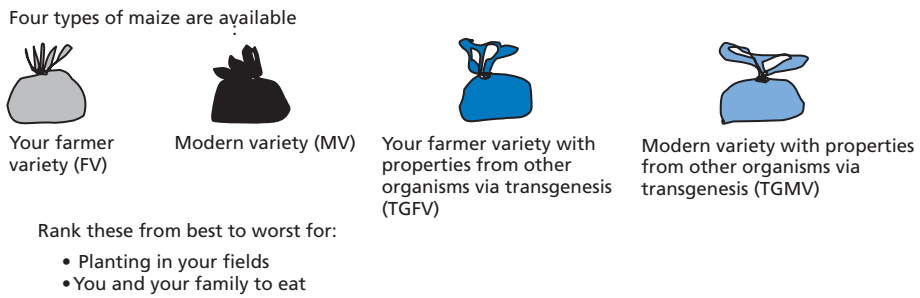
Thus, experiences under diverse circumstances can result in local interpretations of the model, by either farmers or scientists, which can be sources of learning for both scientists and farmers (Cleveland and Soleri, 2002b). When FK differs from that presumed by plant breeders' interpretation of the model, we should try to understand the difference in terms of the specific genotypes and environments each works with, as well as other factors in their experience.

14.1.4 Methods for understanding farmers' knowledge and practice

The best starting place for collaboration may be simple interviews with a representative random sample of households. Such interviews can provide insights critical for collaboration. There are many resources available describing how to conduct such interviews (e.g. Cleveland and Soleri, 1991) and analyse them (e.g. Stern *et al.*, 2004). The key requirements are that: (i) the sample is representative of the human population with which you are working, possibly requiring a stratified sampling approach, based for example on gender of farmers, household socio-economic status, or dominant soil type on farms; (ii) people conducting the interviews are consistent, respectful, open and primarily listen to and document farmers' answers and comments; and (iii) questions are relevant for understanding and collaboration.

In addition to simple questions to elicit basic descriptive data (household size, number working in farming, area sown to each crop, sources of planting seed, yields, etc.), methods such as scenarios and ranking exercises may use hypothetical varieties to better understand farmers' theoretical knowledge, or actual varieties they are familiar with for insights into specific experiences and observations (Crossa, Bellon and Franco, 2002; Soleri and Cleveland, 2005). For example, a scenario using hypothetical maize varieties was created to better understand the G×E interaction most valued by maize farmers in a study in Mexico, Cuba and

FIGURE 14.2
Ranking exercise as presented to farmers

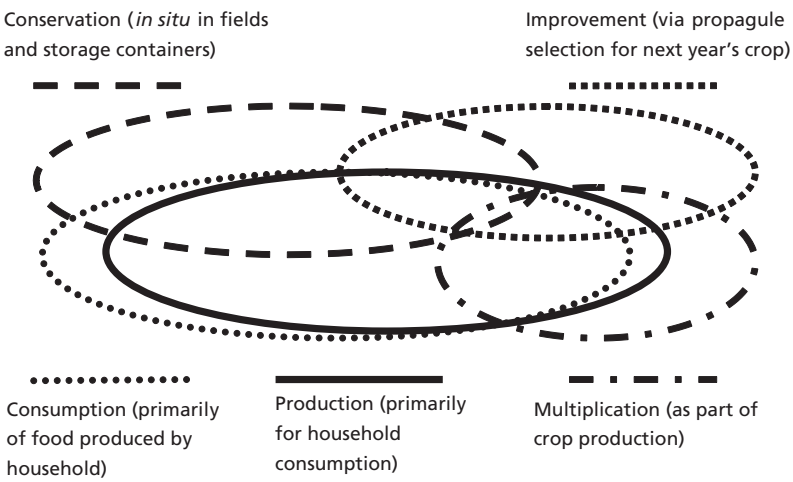


Guatemala (Soleri *et al.*, 2005). When asked to choose between two maize varieties with qualitative G×E response to annual precipitation, 61 percent of farmers preferred a variety with lower yield potential, mean and yield variation ('stable') to a variety with higher yield potential, mean and yield variation ('responsive'). The answer varied, with farmers from more difficult growing environments preferring the stable variety at a significantly greater frequency than those in more favourable growing environments. A similar scenario was created to investigate farmers' attitudes towards some of the possible consequences of pesticidal transgenes in their maize varieties and the evolution of resistance in the pests that it controlled. Some of these consequences were reliance on the formal seed system, a higher seed price and initially high but declining yields over time as pest populations evolved resistance. The hypothetical transgenic variety was not identified as being transgenic when the scenario was presented to farmers. Of those interviewed (n = 334), 70 percent chose a lower yielding but more stable and locally available variety (Soleri *et al.*, 2005). Similarly, an exercise asked those farmers to rank four types of maize: their own FV, a conventional MV they were familiar with, and those same varieties as backgrounds for a transgene: a transgenic farmers' variety and a transgenic modern variety (Figure 14.2). We asked farmers to rank these first as maize seed for sowing in their own fields, and then again as maize grain for their family to eat. The FV and MV represented two seed systems (informal vs formal, respectively) and had different agronomic, storage and culinary characteristics with which farmers were already familiar. Farmers had no previous experience with transgenic crop varieties (TGVs). Providing these four choices allowed us to distinguish farmers' preferences for varieties or genetic backgrounds (FV vs MV) from their preference for a genetic technology (TGV vs non-TGV), an important distinction that is either overlooked or confounded in most research with farmers. TGVs were described neutrally to farmers and they were given a positive example of TGVs with the potential to decrease pest damage.

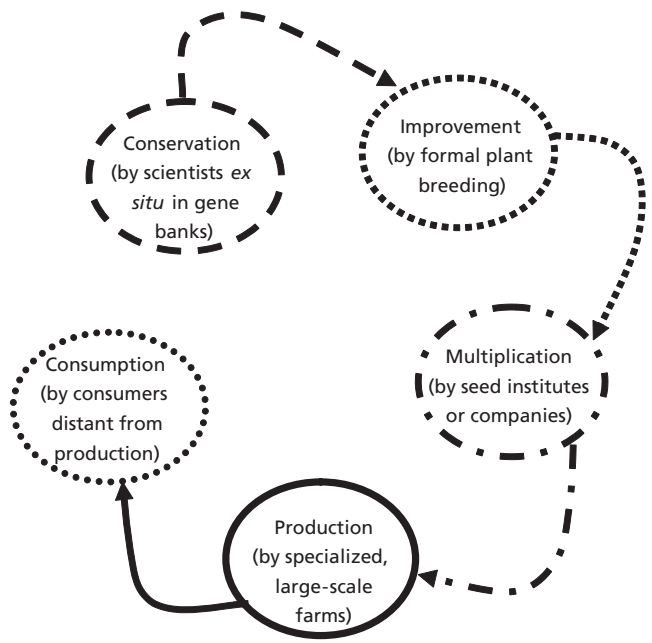
14.2 THE CONTEXT: INDUSTRIAL AND THIRD WORLD AGRICULTURE

Industrial and Third World agriculture are different in important ways in terms of seed and food systems, growing environments and crop genotypes. They are also similar in

FIGURE 14.3
Components of agricultural systems in traditionally-based small-scale and industrial large-scale agriculture



a. Traditionally-based agricultural system: functions integrated in households and communities



b. Industrial agricultural systems: functions separated, specialized, many institutionalized

terms of the basic principles and processes governing these variables and the interactions among them, including the outcome of choice and selection. Better understanding of these differences and similarities, and their relationship to differences and similarities between farmers' and breeders' goals, knowledge and practices, can help to further collaboration between farmers and plant breeders.

14.2.1 Seed and food systems

In industrial agriculture, food production, food consumption, crop improvement, seed multiplication and crop genetic resources conservation are specialized, physically and structurally separated, and farming is often considered to be primarily a business (Lyson, 2002) (Figure 14.3a). In SSTW agriculture these functions are combined within the farm household and community (Figure 14.3b) (Soleri and Cleveland, 2004), as described below. The differences due to separation vs integration of these critical functions in seed and food systems have important implications for decisions about the best ways for farmers and breeders to improve yields and quality traits, and to minimize farmers' risk.

Production

SSTW agriculture is essential for feeding a significant proportion of the world population now, and will probably remain so in the future, even with production increases in large-scale, industrial agriculture (Hazell *et al*., 2007; Heisey and Edmeades, 1999). As mentioned earlier, over 2 billion people live on almost half a billion small-scale farms (<2 ha) in the Third World, including half of the world's undernourished people and the majority of those living in absolute poverty (Nagayets, 2005). Food production relies on household labour, and most households sell some portion of their production in the market, but they are incompletely integrated into these markets (Ellis, 1993). Farmers' production knowledge combines understanding based on theory and empirical observation with values about the social and cultural significance of farming, often focused on FVs (Soleri *et al*., 2002).

Off-farm income is often critical for households' overall survival strategy, and may reduce the importance of on-farm production. Migration of household members, for example, may lead to labour shortage (Narayanan and Gulati, 2002) and to reduced time and other resources devoted to seed selection, conservation of crop genetic diversity or production, and eventually to loss of the knowledge on which they depend (for an example in central Mexico, see Fitting, 2006).

Consumption

Farm families rely on their own food production for a significant proportion of their food, and FVs are valued for traits that contribute to storage, food preparation, taste, colour, texture and specific uses (e.g. maize varieties grown for husks used in tamale production) (Soleri and Cleveland, 2001), or sticky rice FVs used for traditional foods in southern China (Zhu *et al.,* 2003). These specialized uses mean some FVs have high market values.

Improvement

Cultivation in new locations, farmers' changing selection criteria and growing environments were responsible for the tremendous increase in intraspecific crop diversity via mass selection following domestication (Harlan, 1992; Matsuoka *et al.*, 2002) (see Chapter 1, this volume), and all of these continue today. It has been best documented at a local level in vegetatively

propagated crops (Elias *et al.*, 2001), but also in predominantly self-pollinated crops such as rice (Dennis, 1987; Richards, 1986). For cross-pollinating crops like maize, farmers may not be interested in changing quantitative phenotypic traits of their varieties through selection, but rather in maintaining qualitative traits of interest (Pressoir and Berthaud, 2004b), and can do so successfully even in the presence of high rates of gene flow at other loci (Louette, Charrier and Berthaud, 1997; Pressoir and Berthaud, 2004a). Quantitative improvement in such species may more often be sought through choosing new varieties or populations, as discussed below.

Seed multiplication

Farmers do not usually distinguish seed multiplication from food production, although sometimes they plant separate seed multiplication plots, as do some rice farmers in Sierra Leone (Richards, 1986: 138–144). Farmers often save a high proportion of their seed from their own harvests, but often also obtain seed through informal seed systems (Ndjeunga, 2002), and frequently experiment with new seed (Louette, Charrier and Berthaud, 1997), including planting seed obtained as grain (Soleri *et al.*, 2005). The result is extensive gene flow via seed and other propagules, as well as by pollen flow, creating seed systems that are predominantly local and genetically open (Berthaud *et al.*, 2001; Pressoir and Berthaud, 2004a; vom Brocke *et al.*, 2003a).

Conservation

Farmers conserve crop genetic diversity of FVs *in situ* in their fields and storage containers (Qualset *et al.,* 1997). Most *in situ* conservation is done indirectly—perhaps unintentionally—as a result of using or selecting and saving FV seed each year for planting (Louette and Smale, 2000; Soleri, Smith and Cleveland, 2000). This conservation is dynamic in that populations are exposed to changing natural and artificial selection pressures, often creating locally distinct and adapted populations through indirect selection.

Because food production and consumption and crop improvement, seed multiplication and conservation are all carried out within the same crop population, that population will not be optimized for any one function *per se* as it might be in industrialized systems. For example, the value of FV genetic diversity in its local conservation role may in some way be in conflict with the genetic composition 'optimal' for its role as an improved population (Soleri and Smith, 1995). In this sense, farmers' crop populations are similar to semi-natural plant populations that

> *...approach complex equilibria in which overall fitness is as high as the varied demands of differing sites and seasons, complex genetic control and the long term demands of adaptability allow.*
>
> (Simmonds and Smartt, 1999: 91)

For this reason, and because of the value of this farming for production and dynamic conservation, both *in situ* conservation by farmers and *ex situ* conservation in formal gene banks are necessary and complementary. However, for conservation to play a useful role, interaction between farmers and scientists is required, e.g. to ensure that the selection environments in *ex situ* conservation do not result in evolution that makes the population unsuitable for farmers in the event that they require renewal of their seed from outside their communities (Soleri and Smith, 1995). In a similar way, collaboration between farmers and

plant breeders needs to balance the goals of the breeder, which will tend to focus on improving specific traits, with the other functions of the food and seed system.

14.2.2 Growing environments and genotypes

Growing environments and crop genotypes of Third World farmers differ in important ways from those with which most plant breeders and agronomists in industrial countries are familiar. Farms often consist of a number of small, scattered fields with marginal growing environments, i.e. relatively high levels of stress and of temporal and spatial variability. For example, while the average size of maize grain farms in the United States of America in 2003 was 79.2 ha (USDA NASS, 2004), in the southern Mexican state of Oaxaca over 76 percent of maize farms were smaller than 5 ha in 1995 (INEGI, 2001), and in one of the communities in the Central Valleys of Oaxaca where we have worked, the average farm size is 3.7 ha and the average maize field size is 0.8 ha (Soleri, 1999; Soleri, Cleveland and Aragón Cuevas, 2003). In that same Oaxacan community, coefficients of variation of maize yields calculated using triangulation of farmer estimates were very high, averaging 44 percent (Soleri *et al.*, n.d.). Indeed it has been estimated that maize farmers in that area of Oaxaca experience production failure one year in four due to drought (Dilley, 1997). In addition to high levels of environmental variability, other factors contribute to high levels of yield variability and production risk. SSTW farmers typically use low levels of external inputs, and have limited access to government programmes and markets, and limited influence on the policies affecting them (Ellis, 1993; Hardaker, Huirne and Anderson, 1997).

For many plant breeders who work with farmers, this environmental stress and variation mean that selection for improved performance in farmers' environments needs to take place in those environments, and requires re-thinking some of the assumptions of conventional plant breeding (Ceccarelli and Grando, 2002). However, many plant breeders, especially those with little experience with farmers' growing environments, believe that as a general principle selection should be done in optimal environments because there are 'spillover' effects to marginal environments (for discussion, see Atlin *et al.*, 2000; Rajaram and Ceccarelli, 1998). Thus, while plant breeders agree on the basic principles of selection, they can disagree vehemently about how those principals should be applied to farmers' environments (Ceccarelli and Grando, 2002; Cleveland, 2001). One source of such disagreements may be differing interpretations of empirical observations and theories thought to underlie them (see Section 14.2.3).

Farmers often continue to use locally selected FVs, even when MVs produced by the formal plant improvement and seed multiplication systems are available, because FVs may be better adapted to marginal growing environments, and because MVs may be agronomically, culinarily and economically inappropriate (Ceccarelli *et al.*, 1994; Evans, 1993; Heisey and Edmeades, 1999). Farmers value FVs for agronomic traits, such as drought resistance, pest resistance and photoperiod sensitivity, as well as for traits contributing to storage, food preparation, taste, market value and appearance (Smale, 2002). FVs include landraces, traditional varieties selected by farmers, MVs adapted to farmers' environments by farmer and natural selection, and progeny from crosses between landraces and MVs (sometimes

referred to as 'creolized' or 'degenerated' MVs) (Zeven, 1998; FAO, 1996).

FV yields are often much lower in the Third World compared with MV yields in industrialized agriculture, e.g. maize yields in United States of America (~8 t/ha) compared with Mexico (~2 t/ha) (Aquino *et al*., 2001) and Oaxaca (~0.8–1.5 t/ha) (Aragón-Cuevas *et al*., 2006). However, yield stability is often greater with FVs than for MVs grown in the same environments, because MVs often have steep response regression curves, i.e. are highly responsive to marginal environments, as well as optimal ones (Ceccarelli, 1997; Evans, 1993).

An important reason for the higher yield stability of FVs is their higher level of genetic diversity compared with most MVs, presumed to support broad resistance to multiple biotic and abiotic stresses (Brown, 1999). In addition, many centres of origin and centres of diversity for crop species are in the Third World and cultivated primarily by small-scale farmers, thus SSTW agriculture is an important reservoir of genetic diversity in the form of FVs (FAO, 1996). This diversity makes FVs valuable not only for farmers, because they decrease the production risks in marginal environments, but also for the *in situ* conservation of crop diversity as a source of resources for breeding MVs.

14.2.3 Plant breeder knowledge

As outlined above (Section 14.1.3) the basic model of plant genotype-environment interactions are well established and universally accepted by plant breeders. However, many complexities of that model are still not well understood in terms of biological theory (Duvick, 2002), and there continue to be disagreements about the interpretation of the basic model and its implications for practice among plant breeders, such as the effect of selection environment on the range of target environments to which a genotype is adapted (Atlin *et al*., 2000; Bänziger and de Meyer, 2002; Ceccarelli and Grando, 2002).

In specific situations, understanding this basic theory is difficult because a great number of variables affect it, and predictions are hampered by the lack of experimental data and lack of the technologies and resources necessary to gather and analyse them. Plant breeders recognize that their theoretical understanding of plants beyond the basics is limited, and that much plant breeding has been based on intuition and empiricism rather than theory (Duvick, 1996; Simmonds and Smartt, 1999; Wallace and Yan, 1998), although intuition and empiricism are likely to be underlain to a lesser or greater extent by the basic theoretical understanding of genotype x environment relations.

This fundamental biological theory is the same no matter where plant breeding is practised. However, the biophysical, economic and sociocultural variables through which this and other theories work can be quite different. For example, think of the contrast between farmers' fields in marginal environments and plant breeders' research stations, or between national agricultural policy priorities of large-scale efficiencies and increased inputs and production, and farmers' priorities of reducing risk and optimizing crop production as part of a general household survival strategy. Work under a specific set of circumstances may lead to interpretation of theory that is then generalized and broadly applied, without investigating the validity of those interpretations under all circumstances. For example, the fundamental principle that—all else remaining constant—as σ_E^2 decreases, h^2 increases has been interpreted to imply that selection in low σ_E^2 environments provides

the best response for all environments, including ones with high σ_E^2. However, empirical testing has shown this not to be true in many cases (e.g. Ceccarelli, 1996; Ceccarelli *et al.,* 1994, 2003; Comadran *et al.,* 2008); two reasons are that, first, the genes responsible for a quantitative trait such as yield may be different in different environments (e.g. Atlin and Frey, 1990; Atlin, McRae and Lu, 2000; Venuprasad, Lafitte and Atlin, 2007), and, second, h^2 of some important traits may not be entirely obscured by σ_E^2 (Al-Yassin *et al*., 2005) (see Chapter 2, this volume). Working with farmers often requires that breeders test the validity of those interpretations of theory that form the basis of conventional plant breeding. This includes comparing the genotypes and environments and goals for improvement, and testing the assumptions (biological, environmental, economic, sociocultural) on which they are based, and adjusting interpretations of theory, and hence methods used (Ceccarelli and Grando, 2002).

For this reason, farmer–breeder collaboration may often benefit from making a clear distinction between (a) fundamental biological theory, (b) interpretations of fundamental theory, and (c) methods and practice, with 'c' possibly very different depending on whether it is based on 'a' or 'b', or on different versions of 'b'. Many of the disagreements about plant breeding methods for participatory plant breeding (PPB) may grow out of disagreements about differences in the *interpretation* of fundamental biological theory, and disagreements about these interpretations may in turn be based on the belief of proponents that their interpretations of fundamental theory are *not* based on their unique experiences and assumptions, but rather are part of fundamental theory.

Therefore, especially for those biophysical aspects of genotypes and environments that are less well understood in terms of plant breeding theory, PBK may more likely to be based on each person's or institution's specific experiences with the particular environments and crop genotypes they work with, and thus may be less generalizable, and more apt to be influenced by pre-existing knowledge (including values) specific to the plant breeder's social environment. This means that disagreements between farmers and plant breeders, and among plant breeders, could arise even though fundamental genetic and statistical principles remain constant across a range of contexts, because the 'art' of plant breeding is more tied to specific individuals or environments (Ceccarelli and Grando, 2002; Soleri and Cleveland, 2001).

14.2.4 Farmer knowledge

A lack of empirical research and theoretical analysis has contributed to using overly simplified definitions of FK (and often of PBK as well), and the common failure to test the many assumptions underlying these definitions (Cleveland, 2006; Sillitoe, 1998). We can very roughly divide current views of FK into two categories: there are those that see FK as fundamentally different from PBK, and those that see it as fundamentally similar. These views also form the basis of particular advocacy perspectives; generally neither considers the theoretical content of FK.

In the first category, definitions of FK emphasize that it is primarily value-based, comprising intuition and skill, socially constructed, and based on the local social and environmental contexts and culture. According to this perspective, farmer and PBK are seen as fundamentally different, and attempts to explain FK in scientific terms impede true appreciation of FK.

The second category emphasizes that FK consists primarily of rational empirical knowledge, usually focusing on either economic or ecological knowledge. Definitions of FK as economically rational tend to assume that scientists are more rational, and that farmers are risk neutral and their behaviour is based on a desire for profit maximization in the form of high average yields (e.g. Zilberman, Ameden and Qaim, 2007). According to this definition, the role of outsiders should be to facilitate the replacement or modernization of small-scale farming, including replacement of FVs with MVs (Mohapatra, Rozelle and Huang, 2006; Srivastava and Jaffee, 1993). The definition of FK as ecologically rational tends to assume that farmers have detailed, accurate and therefore sustainable ecological knowledge of their environments. The first part of that definition is supported by much empirical data, especially ethnotaxonomic studies of plants and animals, while recognizing variation in distribution of cultural knowledge as the result of factors including age, gender, social status and affiliation, kinship, personal experience and intelligence (Berlin, 1992).

Participatory research has usually been based on the second definition of FK. As a result, the focus in using farmer knowledge has been on the details it can provide in the form of a discriminatory or, most frequently, descriptive tool in PPB. For example, a major survey of 49 PPB projects found that the primary focus was soliciting farmers' descriptions and rankings of selection criteria. For about two-thirds of these projects, "identifying, verifying, and testing of specific selection criteria was the main aim of the research", and 85 percent obtained farmers' selection criteria for new varieties (Weltzien *et al*., 2003: 17–18, 51, 75). The main impact on scientific plant breeding appears to have been "better understanding of new ideotypes based on farmers' experiences, specific preferences and needs" that will affect priorities of formal plant breeding and the "process of formal variety development" (Weltzien *et al.,* 2003: 75).

More recently, using FK of crops as a discriminatory tool has become more common. This has been important in some PPB work, with farmers asked to choose among varieties already released in other areas (e.g. for rice and chickpea; Joshi and Witcombe, 1996), among new and experimental varieties (e.g. for pearl millet; Weltzien *et al.*, 1998), or among segregating populations (e.g. F_3 bulks with barley; Ceccarelli *et al*., 2000), or to select individual plants within segregating populations (e.g. F_5 bulks of rice; Sthapit, Joshi and Witcombe, 1996; and F_4 bulks of rice; Virk *et al*., 2003). When such choice or selection is accomplished using actual plants, plant parts or propagules, analysis of results can reveal farmers' implicit criteria that they may not be able to verbalize easily, if at all (i.e. it may be unconscious) (Louette and Smale, 2000; Soleri, Smith and Cleveland, 2000).

These approaches to understanding FK have made valuable contributions to achieving more effective crop improvement for farmers' conditions. However, the theoretical basis of FK is not usually considered, and rigorous comparisons with PBK have not been carried out, "opportunities rarely develop for interaction between breeders and farmers beyond the survey", with the discussion "driven by the breeders' concepts of the present situation, making it difficult for farmers to express their views in the context of their reality" (Weltzien *et al*., 2003: 51). It may also be difficult for farmers to communicate to outsiders their knowledge that goes beyond description or discrimination. For this reason a

definition of knowledge—both farmer and scientist—as complex, and including values, empiricism, theory and experience is useful (Cleveland, 2006). This definition underlies an approach that starts with basic theoretical knowledge and clearly distinguishes theory from its local interpretation, in an attempt to better understand farmers' choice and selection, and to identify possible bases for substantive collaboration between farmers and scientists. In the rest of this chapter we use this definition to look at two key processes in plant breeding: choice of populations (or varieties) for direct use or for further breeding, and selection of individuals within a population. We focus on our understanding of FK and practice of choice and selection, how farmers and scientists can better collaborate in those steps, and why such collaboration is important.

14.3 FARMER CHOICE AND SELECTION: PAST, PRESENT AND FUTURE

While this chapter is primarily about contemporary farmer and plant breeder choice and selection, a brief look at the broad trends in the past, present and future of crop improvement in relation to farmers will help in understanding the challenges and potential for plant breeding with farmers. This section is not essential for understanding the rest of the chapter, and might be quickly skimmed and used as a reference.

As measured by the rate of desired crop genetic changes achieved by selection, three broad stages have been suggested (Gepts, 2004). Initial rapid progress with domestication was followed by long periods of much slower change as original domesticates spread to new environments and responded to a range of new natural and artificial selection pressures, and with modern plant breeding the rate of change in MVs increased substantially, while most farmers continued as before. There have also been marked changes in crop genetic diversity over time, especially at specific and intraspecific taxonomic levels.

14.3.1 Domestication and subsequent changes in diversity

While domestication resulted in a large decrease in the number of plant species exploited, it was followed by large increases in intraspecific diversity, as FVs evolved as a result of natural and artificial selection in new biophysical and sociocultural environments (Harlan, 1992) (see Chapter 1, this volume). For many of the more widely grown food crops, domestication resulted in evolutionary changes making them genetically distinct from their closest wild relatives today, and most became dependent on humans for reproduction (Harlan, 1992; Simmonds and Smartt, 1999). Exceptions exist, especially among some perennial fruit crops, more accurately described as semi-domesticates, where crops are not the result of selection resulting in *E*, but rather are choices of superior genotypes from among those extant in the wild (for olive, see Baldoni *et al*., 2006, and Breton *et al*., 2006).

Domestication seems likely to have been the result of indirect selection and unintentional direct selection (e.g. when farmers select for large seed size or brittle rachis as a result of their seed collection behaviour; Harlan, 1992), and perhaps some intentional selection for evolutionary change (see Section 14.1.2). However, it is very difficult or impossible to determine the type of selection that resulted in past crop evolution, and experts differ on the type they believe was most important. For example, Allard emphasizes direct, intentional selection,

> *The consensus is that even the earliest farmers were competent biologists*

> *who carefully selected as parents those individuals … with the ability to live and reproduce in the local environment, as well as with superior usefulness to local consumers.*
>
> (Allard, 1999)

In contrast, Simmonds and Smartt (1999: 13) emphasize indirect selection: "the art of cultivation is perhaps the peasant's most potent contribution."

Similar to studies based on archaeological data, results of molecular analyses support the hypothesis that farmers' selection has been successful in achieving evolutionary change for traits in the 'domestication syndrome' that might be indirectly or unintentionally favoured because of agronomic superiority (see Chapter 1, this volume). There is also evidence that farmer selection has been a powerful force for evolutionary change based on other preferences as well. For example, three major genes involved in starch metabolism in maize were found to have unusually low genetic diversity compared with its closest wild relative (teosinte, *Zea mays* subsp. *parviglumis*), which is strong evidence of selection for specific processing and culinary qualities important for the primary manner in which maize has been consumed in its regions of origin and diversity (Whitt *et al*., 2002). In addition, three other loci contributing to sweet maize grain phenotypes showed low diversity (resulting from strong selection) in only certain varieties in particular locations, evidence of further specialization in the non-agronomic selection pressures farmers have exerted on maize (Olsen *et al*., 2006; Whitt *et al*., 2002). Similarly, it appears that strong directional selection for sticky, glutinous grain quality resulted in a selective sweep affecting an area over 250 kb long that includes the locus coding for this quality (low amylase production) and other linked loci. The presence of this sweep distinguishes the sticky rice favoured by upland northeast Asian peoples from the non-glutinous rice varieties used by other Asian groups, and presumably would be among their fundamental choice criteria, perhaps as an adaptation for eating with chopsticks (Olsen *et al*., 2006).

Increasing evidence for a number of crops suggests that domestication could have occurred over short periods relative to the ~12 000 years that crop plants have been cultivated (Gepts, 2004). Domestication syndrome traits often appear to be determined by a small number of genes with large effects, suggesting that domestication could proceed relatively rapidly. For example, Paterson *et al*. (1995) found a small number of quantitative trait loci (QTLs) coding for the domestication syndrome traits of seed size, photoperiod sensitivity of flowering, and brittle rachis in taxonomically distinct cereals with diverse centres of origin (sorghum, rice and maize). In common bean (*Phaseolus vulgaris* L.), control of the domestication syndrome involves genes that have a large effect (>25–30 percent) and account for a substantial part of the phenotypic variation observed (>40–50 percent) (Koinange, Singh and Gepts, 1996). Simulations based on sequence variations at loci coding for biochemical or structural phenotypes in maize and its close and distant relatives have estimated that domestication could have taken from 10 (Eyre-Walker *et al*., 1998) to between 315 and 1 023 generations (Wang *et al*., 1999). In addition to selecting for characteristics of the 'domestication syndrome', especially in cereals and small pulses (Harlan, 1992), domestication in sexually propagated crops may have resulted in increased autogamy and therefore homozygosity, expressed

phenotypically in greater trueness to type in a population over generations. In contrast, some vegetative propagation may have selected for heterozygosity (via heterosis) and therefore for allogamy, as contemporary evidence suggests for cassava (Pujol, David and McKey, 2005).

The genetic changes that define crop domestication are inextricably linked with changes in selection pressure. These pressures are not only exerted by direct human selection of propagules for planting, but perhaps more often with the differences in selection pressures created by human modification of growing environments (Figure 14.1). In southeast China, for example, evidence for the earliest cultivation of both wild and domestic rice (~7 700 BPE) suggests that this occurred where farmers were intensively managing coastal wetlands with fire to control vegetation and bunds to control flooding, and increased nutrient concentration in fields (Zong *et al*., 2007). Bringing wild plants into human modified environments, such as compost heaps near houses, as well as exchange of seeds and other propagules, also facilitated domestication via hybridization, as with *Leucaena* in southern Mexico, and probably with two other important domesticates from that region, agave (*Agave* spp.) and prickly-pear cactus (*Opuntia* spp.) (Hughes *et al*., 2007). Domestication generally decreased the fitness of plants in natural environments, and made them more dependent on humans and human-managed environments.

The geographical spread of domesticated crops led to great varietal diversification as a result of the increase in diversity of natural and artificial selection pressures encountered, followed by choice among preferred populations. It is generally assumed that simple mass selection by farmers working in combination with local natural selection contributed to the large amount of intraspecific diversity that evolved following domestication:

> *Probably, the total genetic change achieved by farmers over the millennia was far greater than that achieved by the last hundred or two years of more systematic science-based effort.*
>
> (Simmonds and Smartt, 1999: 12).

14.3.2 Modern, scientific plant breeding

Farmer and plant breeder crop improvement began to be separated about 200 years ago in "technically advanced temperate countries" (Simmonds and Smartt, 1999: 12) with the beginning of specialized, amateur breeding. The widespread acceptance of evolution and the rediscovery of Mendel's research after 1900 eventually led to modern scientific plant breeding, based on a combination of Darwinian evolution, Mendelian genetics and biometry (Fitzgerald, 1990; Provine, 1971), with modern plant breeders considering themselves 'applied evolutionists', whose goal is to develop plant varieties better adapted to growing environments, measured primarily as increased yield (Allard, 1999).

Farmers and formal plant breeders continued to collaborate at this time, for example in making crosses and selections in maize breeding in the United States of America (Fitzgerald, 1990; Schneider, 2002). But as the importance of evolutionary theory in plant breeding increased in comparison with empirical heuristics, the economic importance of plant breeding increased and came to dominate formal plant breeding by professional plant breeders. Simultaneously, the farmer's role in crop improvement in industrial countries decreased, for example in the United States of America (Fitzgerald,

1990; Kloppenburg, 1988) and Switzerland (Schneider, 2002). Plant breeders' concepts subsequently developed independently of farmers' concepts, effectively separating the formal from the informal systems of crop improvement and seed multiplication. When farmers are involved by contemporary plant breeders in their work it has generally been limited to the stage of evaluating and choosing among plant breeders' populations or varieties in their fields (Duvick, 2002).

14.3.3 Biotechnology

Advances in genetics and molecular biology have led to developments in biotechnology that have dramatically enhanced the ability to understand and manipulate plant genomes. Functional genomics has elucidated the relationship among genetic components and to phenotypes; marker assisted selection (MAS) has increased the efficiency of breeding for specific traits; and genetic engineering has made it possible to transfer genes from almost any organism into a crop species. When these genes come from a different species the process of transformation is called transgenesis, and the resulting crop variety a genetically engineered (GE) variety, genetically modified organism (GMO) or, most accurately, a transgenic crop variety (TGV).

TGVs are a rapidly growing agricultural technology, with the area planted increasing by 9.4 percent from 2007 to 2008, to over 125 million hectares (James, 2006, 2008), or over 9 percent of cultivated land globally (calculated from FAO, 2007, 2009). Currently grown TGVs are primarily targeted to industrial agriculture and designed to enhance yield and net profit for farmers by directly reducing pest damage or facilitating herbicide use. Globally, most of the area planted to TGVs is in large-scale industrial agriculture, and is expanding in the Third World. Of the 23 countries growing TGVs in 2007, 12 were 'developing' countries, and estimated to account for 43 percent of the area planted and 90 percent (11 million) of the farmers growing TGVs. Of these, 99 percent (10.9 million) were in China and India, growing mostly Bt cotton (James, 2006, 2007). Currently, TGVs of food crops for Third World farmers are either planned, being developed, in field trials, or approved and in production.

TGVs are currently being promoted by development organizations, governments and corporations as the key to increasing production and income and reducing hunger and malnutrition in SSTW agriculture (FAO, 2004; Rockefeller Foundation, 2007; World Bank, 2007). However, the focus on TGVs to improve Third World agriculture is very controversial (Abate *et al*., 2008; Stokstad, 2008). A number of studies, mostly by economists and of Bt cotton, maize and rice, have concluded that farmers readily adopt TGVs because they increase yield and income, reduce pesticide applications or improve farmer health (Gouse *et al*., 2006; Huang *et al*., 2003, 2005; Morse, Bennett and Ismael, 2006; Qaim and Zilberman, 2003). Other studies have found that adoption may be the result of fads (Stone, 2007) or a lack of freedom to choose (Witt, Patel and Schnurr, 2006), and that higher yields and reduced pesticides may be reversed after several years due to the emergence of secondary pests (Wang, Just and Pinstrup-Andersen, 2006). Others have suggested that the net benefits of TGVs may not be as great as those of alternative improvements in agriculture (e.g. Uphoff, 2007). The potential ecological and genetic effects of TGVs and transgene flow into non-TGV crop or wild or weedy populations, especially in Third

World agriculture, are not well understood (Ellstrand, 2003b; Heinemann, 2007; NRC, 2002; Snow *et al*., 2005).

The spread of biotechnology has also resulted in unintentional transgene flow, including into centres of diversity, e.g. maize transgenes documented in Mexican FVs (Alvarez-Morales, 2002; Pineyro-Nelson *et al*., 2009; Serratos-Hernández *et al.*, 2007). Such transgene flow can be difficult to prevent (NRC, 2004), the early stages of transgene flow to FVs are extremely difficult to monitor (Cleveland *et al.*, 2005), and the effects may often be irreversible (Ellstrand, 2003a). Potential effects of transgene flow on FVs and farmers are both positive and negative, and will require risk analysis and evaluation specifically adapted to each location – crop combination within the Third World (Cleveland and Soleri, 2005; Soleri, Cleveland and Aragón Cuevas, 2006). Transgenes can introduce novel forms of diversity into the crop populations being selected upon by farmers and plant breeders, but there is no reason to expect that farmers will be able to retain, discard or manipulate them any differently from other genes.

14.3.4 Privatization

In the early 1980s, some countries and farmer support groups sought to do away with all intellectual property rights (IPRs) in crops, establishing 'farmers' rights' to all crop genetic resources, but this move was defeated by the United States of America and other industrial nations (Fowler, 1994), and private rights in plants and other living organisms now dominate, with industrial patents leading the way (Atkinson *et al*., 2003). Farmers were left with having to defend themselves from the advances of an IPR system in plants designed by industrial nations and corporations, a system that generally does not recognize farmers' traditions or current needs (Cleveland and Murray, 1997).

Much plant breeding has moved from the public to the private sector (Frey, 1996) and thus selection criteria are increasingly vulnerable to being dominated by private profit motives rather than public good motives (Simmonds, 1990), which is especially evident for TGVs. The major share of agricultural biotechnology processes and products are controlled by private multinational corporations with little incentive to develop TGVs most appropriate for Third World farmers who cannot afford to pay the premium for TGV seed (CGIAR, 2006; World Bank, 2007: 178).

Similarly, there is increasing concentration in the seed sector, which potentially reduces competition and limits the kinds of crops and crop varieties produced and made available. The largest seed companies control an ever larger proportion of the seed market; according to one estimate, between 1997 and 2004 the companies with the largest sales increased their market share from 27 percent to 33 percent, and in 2004 the top four companies owned 38 percent of biotechnology patents (World Bank, 2007: 135–136).

The drive to globalize industrial-world IPRs in plants has been intensified as a result of pressure from agricultural biotechnology corporations (Graff *et al*., 2003; Shorett, Rabinow and Billings, 2003). This means that as patented TGV crops and their transgenes move intentionally or unintentionally around the world, so could the rights of the companies who own them. Movement of transgenes into non-transgenic crop populations, whether producing a net benefit for the farmer or not, makes farmers vulnerable to IPR claims from the technology developer. In the United States of America and many other industrialized countries, patent

holders have rights to seek damages from farmers who end up with patented genes in their crops, even though farmers do not want them, and do not know they are there (Janis and Kesan, 2002). The World Trade Organization (WTO) seeks worldwide uniformity of laws for IPRs in plants and plant DNA to facilitate global enforcement, and many Third World countries have adopted the industrial world model (UPOV – International Union for the Protection of New Varieties of Plants) while others have adapted their national laws to protect small-scale farmers (World Bank, 2007: 167). The spread of IPRs and coupled economic control of agricultural biotechnology means that Third World farmers and the nation states they live in will have a difficult time gaining meaningful control of the means to intentionally create TGVs more suited to their own needs, if this is the path they choose. As a result, most organizations promoting TGVs more suited to Third World farmers are advocating public-private partnerships (CGIAR, 2006; FAO, 2004; World Bank, 2007), yet it is not clear how farmers' rights will fare in this collaboration, and they are not being rigorously addressed.

While most corporations deny they would enforce their IPRs against Third World farmers, there are no guarantees. In addition to transgenes, control of local farmers' crop genetic resources, and the traditional names and other cultural property that go with them through industrial IPRs, can legally and economically prevent local people themselves from reaping potential benefits in a global marketplace increasingly interested in traditional crops and foods (Soleri *et al*., 1994). There are already cases of this, some of which are being challenged (Pallotini *et al*., 2004).

14.3.5 Sustainability and farmer-scientist collaboration

The search for sustainability provoked by negative environmental impacts of agriculture (Matson *et al.*, 1997; NRC, 2002; Tilman *et al.*, 2002), and its genetic vulnerability (NRC, 1991) has led to the incorporation of more genetic variation within and among varieties by the formal crop improvement system (Cooper, Spillane and Hodgkin, 2001; Ortiz *et al*., 2007). It has also encouraged a re-evaluation of G×E in crops and how best to exploit this for farmers' conditions (Ceccarelli *et al*., 1994, 2001). The impact on farmer selection will be in the greater intraspecific and intravarietal diversity deployed in formally developed varieties, and greater interest in that system for breeding goals more similar to those of farmers. Part of the interest in sustainability (environmental, economic and social) has led to collaboration between farmers and scientists.

Participatory or collaborative plant breeding is attempting to reverse the separation of farmers and scientists and improve the outcomes of choice and selection in farmers' terms (Cleveland and Soleri, 2002a; PRGA, 2004; McGuire, Manicad and Sperling, 2003; Weltzien *et al*., 2003). To that end, the next sections focus on understanding farmers' choice and selection, and thereby enabling farmers and scientists to work more closely and productively in improving the crops they grow and depend upon.

14.4 CHOICE OF GERMPLASM

It is important for plant breeders to understand how and why farmers choose varieties of their crops, because farmer choice will ultimately determine whether a new or improved variety will be useful. In this section we consider choice based on perceived

risk and yield stability, and on other factors, including quality traits.

Just as there are factors favouring the inclusion of more than one variety in a farmer's crop repertoire, there also are factors limiting the number of varieties chosen. These include farmers' resources, growing environments and crop reproductive biology, among other possible factors. Additionally, if crop varietal diversity is maintained at a community instead of household level, then farmers may not feel the need to maintain some varieties themselves each year, even though they consider those varieties to be part of their varietal repertoire and intend to grow them in the near future (for the case of rice, see Dennis, 1987).

14.4.1 Varietal choice, yield stability and risk

In much of the past plant breeding for SSTW farmers, it was assumed that high yielding varieties selected in more optimal environments would outyield FVs in farmers' environments (Ceccarelli *et al.,* 1994; Ceccarelli, Grando and Booth, 1996). If farmers did not adopt these varieties it was assumed that they were ignorant of how to improve their growing environments (Aquino, 1998), or if they could not afford to do so, it was assumed that they should get out of farming. Consideration of risk provides a different understanding of farmers' varietal choices and other practices. In the conventional economic model, a risk-neutral farmer would only grow the one variety that gives the highest profits per unit area (Smale, 2002). However, many small-scale farmers in marginal environments are risk averse (Anderson and Dillon, 1992; Soleri *et al*., n.d., 2008), and spatial environmental variation increases the likelihood of cross-overs in varietal performance (qualitative G×E; see Section 14.5.1, below) between farmers' fields, or even within a field (Soleri *et al*., 2002). Variation in time is also large: in the semi-arid tropics, seasonal and annual rainfall is highly variable, and even in years with adequate total rainfall, rains may arrive late, end too early, stop for a period or be too heavy during flowering or harvesting. Therefore farmers may often grow two or more varieties of many crops, each with distinct agronomic characteristics presumably "as a measure of insurance against vagaries of the weather, diseases, or pests" (Doggett, 1988).

Understanding farmers' choice can provide valuable insights for scientific plant breeders. In response to climate change in the form of the southern movement of isohyets, policy-makers in Mali argue that improved short-cycle varieties are a critical part of stabilizing the country's volatile cereal production (Dembélé and Staatz, 2000). One result is that both sorghum breeders and farmers in southern and central Mali look north for shorter cycle varieties. Interviews in four villages in the Upper Niger River valley zone of Mali found the most common reason for adoption of the three most popular sorghum varieties was early maturity (Adesina, 1992). However, since in good rainfall years long-cycle varieties generally have higher yields (Adesina, 1992) and are rated higher for quality (Ingram, Roncoli and Kirshen, 2002), farmers do not give these up entirely. Their choices thus increase the number of varieties in their repertoires, although the net impact on genetic diversity has not been investigated. Another study in Mali of farmers' choices among their traditional sorghum varieties in terms of one or more than one variety, and short-cycle or long-cycle varieties, found that farmers make these choices in an effort to optimize outputs in the face of variation in the growing environment and in

availability of human-managed inputs, such as labour and tools. For example, better rains in 2002 compared with 2001 appear to be a major factor in the general shift toward a greater number and longer cycle length of varieties, with 60 percent of farmers adding varieties between 2001 and 2002 (Lacy, Cleveland and Soleri, 2006).

The need for research on farmer choice and risk is also illustrated in the case of potato in the Andes (Zimmerer, 2002). An emphasis on potato varieties with large tubers because farmers prefer the higher yield of these varieties would ignore the fact that poorer farmers actually select small tubers for planting because they can reduce the amount of potential food used for planting material. An implication is that the varieties poor farmers would actually choose to plant may be quite different from that anticipated by breeders, indicating changes were needed to make improvement programmes more relevant for those farmers' needs.

These and other studies suggest that crop improvement programmes need to specifically target farmers' growing environments and needs, and use local germplasm as the basis for this (Ceccarelli and Grando, 2002). They indicate the importance of plant breeders supporting varietal portfolios (Ceccarelli *et al*., 2003; vom Brocke *et al*., 2003a; Weltzien *et al*., 2003) available through farmer-to-farmer exchange as an alternative to the development of a small number of varieties for large-scale adoption. In addition to decreasing farmer risk, this strategy also supports conservation of crop genetic diversity *in situ* (Ceccarelli, Grando and Baum, 2007). However, there is also some evidence that MVs developed through participatory varietal selection can replace existing FVs, as with wheat in South Asia (Ortiz-Ferrara *et al*., 2007). When environmental variation is minimal, there may be little incentive for farmers to maintain FVs while adopting MVs in order to reduce risk due to qualitative G×E (Virk and Witcombe, 2007). Clearly, the diversity outcome of locally focused improvement programmes will depend on the specific situation.

14.4.2 Other factors influencing choice

Farmers may also choose more than one variety because of their different quality traits. For example, interviews with 599 Nigerian farmers supported the conclusion that they grow both long-cycle and short-cycle cowpea varieties: short-cycle for food grain and long-cycle for feed during the dry season when other fodder sources are scarce (Abdullahi and CGIAR, 2003). Some maize farmers in Oaxaca, Mexico, maintain varieties specifically for their coloured husks or tassels because of their aesthetic qualities, e.g. coloured husks used to wrap tamales impart their colour to them (Soleri, field notes, 1996–1999), and families who make the traditional beverage *tejate* maintain more varieties of maize, using them in its preparation (Soleri, Cleveland and Aragón Cuevas, 2008).

The number of varieties grown by farmers may also be influenced by seed source and social variables (David, 2004). In a study of Mexican maize farmers, choice of total number of varieties grown was related to household seed source. Households planting mostly their own seed chose an average of twice as many varieties in comparison with those households that obtained all their seed from non-household sources (Louette, Charrier and Berthaud, 1997). In a review of field research on farmer crop genetic resources, wealth was a common indicator for producers who cultivated more varieties compared with resource-poor producers (Jarvis *et al*., 2000). The choice of total number of sorghum varie-

ties may be significantly related to ethnicity, as in one area of the United Republic of Tanzania, where migrant Gogo farmers from a traditional sorghum-growing region grow more than twice the number of varieties as migrant groups from maize-growing regions (Friis-Hansen and Sthapit, 2000).

14.5 SELECTION

Given the historical background outlined earlier, including the emphasis on selection as practised by scientists, we now discuss the concept and process of selection, emphasizing the contexts and perspectives of farmers. We begin by reviewing research on farmer understanding of heritability and G×E, two fundamental concepts in selection.

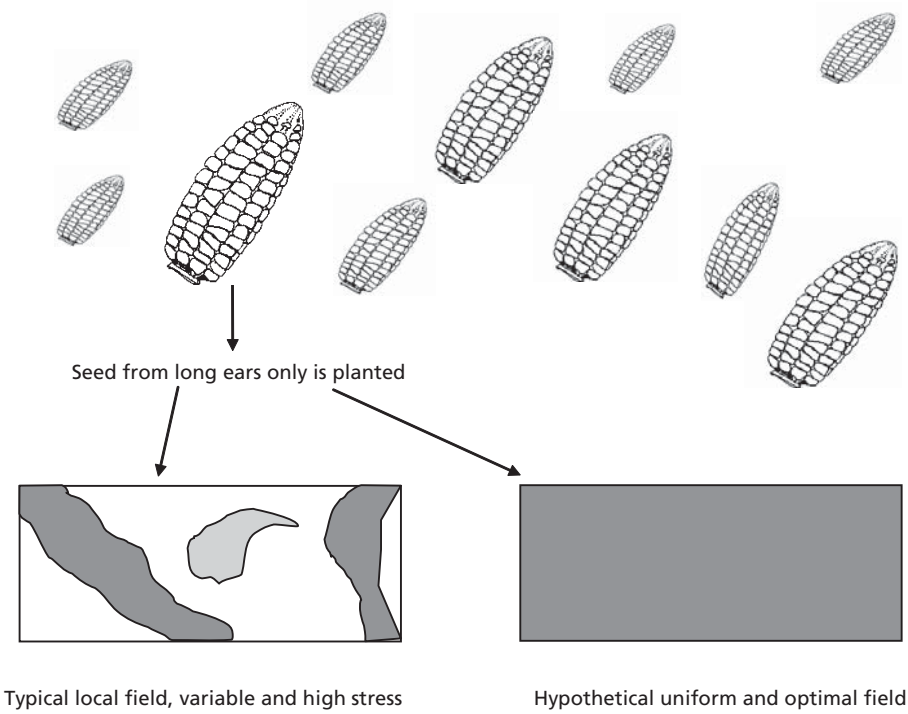
14.5.1 Farmer understanding of heritability and G×E

Heritability (h^2) is a key determinant of genetic response (R) (see Section 14.1.3). One of the main factors that decreases h^2 is environmental variability (σ_E^2). Another important and related element affecting the outcome of selection is G×E. Interpretation of G×E will influence plant breeders' approaches to developing and improving crop varieties and their choices of how many and which varieties will be released across agricultural environments (Cooper and Hammer, 1996). For these two important elements that affect the results of selection, experience as well as goals will influence the knowledge of farmers and plant breeders and how each responds to variations in h^2 and G×E in their crop varieties and growing environments.

In comparative research on farmers' concepts of h^2, farmers were presented with scenarios about both high and low h^2 traits (Figure 14.4, Table 14.1). The goal was to determine if farmers noted the contribution of σ_E^2 and σ_G^2 to σ_P^2, and if they distinguished between high and low h^2 traits in their major crop. The first null hypothesis was that there was no difference in distribution of farmers' responses concerning consistency between parent and progeny phenotypes in a typical, variable environment and in a hypothetical, uniform environment for (i) relatively low h^2 traits, and (ii) relatively high h^2 traits. This hypothesis was rejected for low h^2 traits, but accepted for high h^2 traits (most farmers anticipated no change in phenotype regardless of environment), suggesting farmers see little or no contribution of genotype to σ_P^2 for low h^2 traits, and the opposite for high h^2 traits. The second null hypothesis, that farmers' responses indicate no perception of differences in h^2 for relatively low and high h^2 trait expression in a variable environment, was also rejected, supporting the conclusion that farmers do perceive differences in h^2 of traits. Thus, most farmers distinguish between high and low h^2 traits, and consciously select for the former, while often considering it not worthwhile or even possible to seek $R > 0$ for the latter, especially in cross-pollinated crops (Soleri *et al*., 2002). Given farmers' experiences and the tools and methods available to them, the role of σ_G^2 in low heritability traits is obscured by the σ_E^2 in their growing environments. Similarly, Ceccarelli (1996) argues that plant breeders' lack of experience with growing environments as stressful and variable as those of farmers has obscured plant breeders' ability to perceive qualitative G×E in some MVs between farmers' environments and the more favourable ones they are accustomed to.

To understand farmers' perceptions of spatial G×E interactions we used a scenario with two genotypes originating in contrasting growing environments at three

FIGURE 14.4
Sample scenario used to elicit farmers' knowledge of heritability



For each field we asked, *"What length will the ears that grow in this field be? The same as or different from the ears from which the seed was taken?"*

This scenario, used with farmers in Cuba and Mexico, is about a maize trait with low average heritability (ear length). We asked farmers what the ear length of the next generation would be in a typical relatively variable, stressful field and a hypothetical uniform, optimal field if seed from long ears only was planted. Similar scenarios were used for low and high heritability traits for crops in the five sites in study (see Table 14.1).

TABLE 14.1
Understanding farmers' perceptions of heritability

Location, crop	**Null hypothesis #1:** For traits with relatively low or those with relatively high h^2, distribution of farmers' responses is the same whether scenarios depict typical or optimal environments, i.e. farmers do not see a contribution of environment (Env) to phenotype		**Null hypothesis #2:** In scenarios depicting a typical environment, distribution of farmers' responses is the same for traits with relatively low or those with relatively high h^2, i.e. farmers do not see a difference in h^2 between traits
	(a) Low h^2 trait across typical and optimal Envs	(b) High h^2 trait across typical and optimal Envs	Low v high h^2 traits in typical, variable Env
Cuba, maize	Ear length*	Husk colour	Ear length v. husk colour*
Mexico, maize	Ear length*	Tassel colour	Ear length v. tassel colour*
Mali, sorghum	Panicle weight*	Glume colour*	Panicle weight v. glume colour*
Syria, barley	Plant height*	Seed colour	Plant height v. seed colour*
Nepal, rice	Plant height*	Seed colour	Plant height v. seed colour*

* Hypothesis rejected, Fisher's exact test, $P<0.05$
Based on Soleri *et al.*, n.d.

levels: between locations, between fields in one location, and between places in one field (Soleri *et al*., n.d., 2002). The results indicated that farmers (n = 208) perceive inter- (57 percent) and intra- (30 percent) location G×E for their major crop, though far fewer at the latter level. G×E within a field (18 percent) was noted mostly, though not exclusively, by those growing self-pollinated crops, and especially those working at a small scale with intimate knowledge of within-field soil and moisture variations (e.g. rice farmers in western Nepal). Similarly, 37 percent of farmers responded that a qualitative G×E interaction could occur in their crop due to temporal environmental variation in the form of annual precipitation. In the presence of qualitative G×E, perceptions of the best genetic strategy may differ and be informative about the needs of particular groups or regions. As mentioned in Section 14.4.1 above, farmers tended to favour yield stability over high yield when choosing among varieties in the face of qualitative temporal G×E. This choice was significantly more frequent among farmers in more difficult environments compared with more favourable environments.

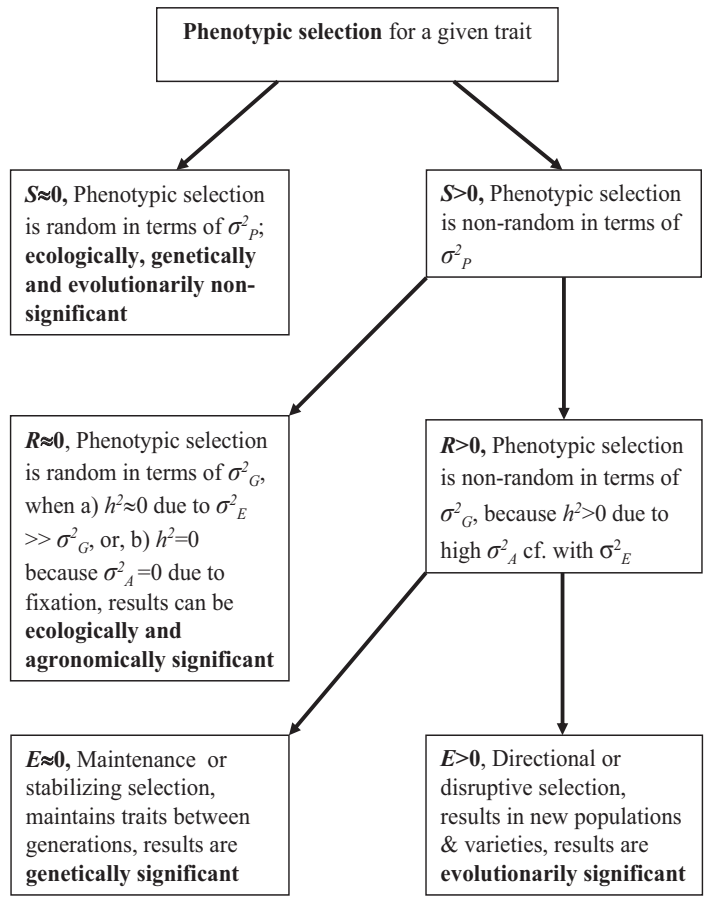
14.5.2 Farmers' selection goals

If plant breeders misunderstand what farmers are and are not attempting to accomplish with their selection practices, it can limit the potential for meaningful collaboration and lead to inappropriate investments of scarce time and resources. Such misunderstandings have grown out of the historical process of separation of farmers' and plant breeders' work (Cleveland and Soleri, 2007).

Just as early evolutionary biologists looked to breeders for empirical demonstration of results of selection that illuminated evolution, breeders looked to farmers for their applied knowledge and practice that produced practical results in the form of new varieties, as in the early commercial development of maize in the United States of America (Wallace and Brown, 1988: 87–90). With the increased importance of formal science in plant breeding compared with empirical heuristics, and later as plant breeding moved from the public to the private sector (Kloppenburg, 1988), plant breeders began to eliminate farmers from their work (e.g. Schneider, 2002). Plant breeders' and farmers' practices and concepts subsequently developed independently of each other, effectively separating the formal and informal systems of crop improvement and seed multiplication, with plant breeders coming to dominate: "a trend that has been at least locally apparent for 200 years" (Simmonds and Smartt, 1999: 13). Plant breeders focused on modern varieties widely adapted to more optimal, more intensively managed environments, while many traditionally-based farmers in relatively marginal environments continued to focus on traditional, specifically adapted varieties for their diverse, more marginal growing environments (Ceccarelli and Grando, 2002; Cleveland, 2001). When contemporary plant breeders involve farmers in their work, it has generally been limited to the stage of evaluating the plant breeders' populations or varieties in the field (Duvick, 2002), i.e. choosing among different populations or varieties, not selecting among different plants to genetically change existing populations or varieties.

Today, many modern plant breeders consider themselves to be 'applied evolutionists', whose goal is to develop plant varieties better adapted to improved growing environments, with adaptation measured primarily as increased yield

FIGURE 14.5
Phenotypic selection classified according to outcome of selection



See text for key to symbols.

(Allard, 1999: 49). Their emphasis in selection is on achieving directional, multi-generational, micro-evolutionary change. This makes sense given the organization of industrial agricultural systems (see Section 14.2.1 above, and Figure 14.3). It also means that plant breeders often view farmers' selection of seeds or other propagules for planting as a form of mass selection for heritable traits, the process that is assumed to account for crop domestication and for the ensuing proliferation of crop varieties. It also means formal plant breeders tend to judge the efficacy of farmer seed saving in terms of applied evolution, i.e. the same criteria they apply to their own work, and assume that farmers use these criteria as well.

In the following sections we describe phenotypic selection by farmers organized in terms of possible outcomes: longer-term (multi-generational) genetic change

or micro-evolution (E) [hereafter referred to as 'evolution', in the sense of multi-generational change in the context of agricultural crops, not in the larger biological sense of speciation]; inter-generational genetic change or response (R); and within-generation phenotypic differentiation (S, selection differential) (see Section 14.1.2 above, and Figure 14.1). Where possible, we also discuss farmer goals for selection, although many studies of farmer selection that document genetic or agronomic effects do not document farmers' goals (and vice versa). Note that, regardless of goals, the outcomes of farmer selection can be varied, as depicted in Figure 14.5.

14.5.3 Selection for evolution

The clearest evidence for contemporary farmer selection for evolution is in species that are normally propagated clonally. Some Andean potato farmers search their fields for volunteer seedlings resulting from spontaneous hybridization as a way to diversify their production (Zimmerer, 1996: 201). For example, indigenous South American farmers intentionally incorporate cassava seedlings into recognized varieties, resulting in increased heterogeneity within varieties (Elias *et al.*, 2001; Pujol, David and McKey, 2005). Farmers also select the largest volunteer seedlings, which results in increased heterozygosity as a result of the most heterozygous plants also being the largest, and therefore the least likely to be eliminated during early weeding, although farmers' goals for this selection are unclear (Pujol, David and McKey, 2005).

In seed-propagated species that are predominantly self-pollinated, compared with cross-pollinated species, it is relatively easy to make and maintain evolutionary changes by selecting from among the segregating F_1 plants or those of later generations, resulting from limited spontaneous cross-pollination. Experimental evidence from Syria shows that farmers could efficiently select among over 200 barley entries (fixed lines and segregating populations), with results in terms of yield potential that equalled, and in one case exceeded, selections by plant breeders in the same environments (Ceccarelli *et al.*, 2000). These findings indicate that farmers have developed selection criteria for identifying high yielding phenotypes that are just as effective as those used by breeders, and more effective in the growing environments typical of those farmers' own fields (Ceccarelli and Grando, 2007).

It is much more difficult to effect evolutionary change in predominantly cross-pollinated, seed-propagated species, especially for quantitative traits with low heritability. However, as described earlier, farmers can discriminate between low and high heritability traits, and use this as a basis for decisions about selection (Soleri *et al.*, 2002). Farmers in Oaxaca, Mexico, often select maize seed with the goal of changing or creating populations with preferred, highly heritable traits, like kernel, tassel and husk colours for culinary and aesthetic reasons (e.g. maize varieties selected for the beauty of their purple tassles) (Soleri and Cleveland, 2001), while the majority of these same farmers see no possibility of changing the key trait of yield, which has low heritability, as discussed below (Soleri and Cleveland, 2001). There is evidence that farmers in central Mexico have selected for and maintained a new landrace, based on seed and ear morphology, among segregating populations resulting from the hybridization of two existing landraces (Perales, Brush and Qualset, 2003). In Rajasthan, India, there is evidence based on research with pearl millet that farmers use

mass selection for low heritability traits in cross-pollinating species with the goal of making directional change in their varieties (Christinck, 2002: 126; Vom Brocke *et al*., 2002). This research also documented farmers' intentional introgression of modern with traditional varieties of pearl millet, and subsequent selection, resulting in increased genetic variation and long-term directional change (*E*) in selected traits, such as growing period (Christinck, 2002: 123; vom Brocke *et al*., 2003a).

However, although it is clear that farmers can understand the principle of phenotypic selection and use it to achieve goals of evolutionary change with different crops, this may not always, or even usually, be their goal, or the result.

14.5.4 Selection for genetic response, but not evolution

Farmers also select with the goal of eliminating changes in phenotypic traits resulting from gene flow or natural or indirect phenotypic selection, i.e. to achieve *R* but not *E*. Best documented are farmers' attempts to maintain varietal ideotypes based on quantitative or qualitative phenotypic traits over time in the face of gene flow (Berthaud *et al*., 2001). Plant breeders can control unwanted gene flow much more effectively in their experimental plots than farmers can in their fields, and in industrial agriculture farmers often buy new seed every year, especially for cross-pollinated crops like maize, eliminating most concerns regarding gene flow.

This type of farmer selection to eliminate changes may contrast with maintenance (stabilizing) selection by plant breeders, which usually has the goal of maintaining yield in the face of changing environments by incorporating new alleles or changing allele frequencies, and may result in new varieties (i.e. the goal is *E*) (Evans, 1993: 313–314). Like plant breeders (Cooper, Spillane and Hodgkin, 2001), farmers also encourage gene flow under some conditions, for example mixing seed from different sources, planting different populations contiguously or in same plot, and by making crosses, as a way of increasing the variation on which to select.

Farmers can be successful in maintaining varietal ideotypes through direct, intentional selection for key traits, especially for highly heritable phenotypic traits, like those that define a variety. This type of selection is probably most important for cross-pollinated crops, such as pearl millet and maize, as discussed below, since it is much more difficult to maintain populations in these compared with clonally propagated and self-pollinated crops. In eastern Rajasthan, India, amplified fragment length polymorphism (AFLP) analysis showed that farmers maintained the ideotypes of distinct introduced pearl millet FVs, even though they have the same name as local FVs, via intentional selection of panicles for their unique phenotypes (vom Brocke *et al*., 2003b). In contrast, farmers in Jalisco, Mexico, regularly mix maize varieties together by classifying seed obtained from diverse sources as the same variety based on ear or kernel morphology and colour, which, together with planting patterns, leads to a 1–2 percent level of gene flow between maize varieties during one crop cycle (Louette, Charrier and Berthaud, 1997). A controlled experiment found that, compared with random selection, farmer selection diminished the impact of gene flow on one FV from contrasting FVs for key varietal traits (kernel rows per ear, kernel width and kernel colour), but did not have any effect on allelic frequencies at 9 polymorphic loci coding for traits invisible or unimportant to

farmers (Louette and Smale, 2000). Farmers stated that they were not interested in changing their varieties, but in maintaining varietal ideotypes, and appeared to be achieving their goal. Research in Oaxaca, Mexico, using microsatellite data supported this finding in terms of the results of farmer selection, although farmers' goals were not investigated. Extensive gene flow and little molecular genetic structure was observed, but the maintenance of significantly different maize populations based on morphophenological traits of interest to farmers persisted (Pressoir and Berthaud, 2004b).

A study in Chiapas, Mexico, found that cultural diversity, as measured by ethnolinguistic groups, was not reflected in maize diversity as measured by isozyme variation, but was reflected in some morphological traits (Perales, Benz and Brush, 2005). The differences observed may have been due to unidentified culturally-based networks or practices that structured these maize populations based on farmer selection for a few critical traits against a background of ongoing gene flow (Perales, Benz and Brush, 2005), as was found in the study in the central valleys of Oaxaca, Mexico, (Pressoir and Berthaud, 2004b), although neither study investigated farmer goals in detail.

14.5.5 Selection for intra-generation phenotypic difference

Although farmers are capable of phenotypic selection that is effective in achieving goals of evolution and genetic response, perhaps the most common goal of farmer selection is not genetic, but solely phenotypic, because most of the time a farmer's primary goal in selecting seed is to obtain good planting material. This often means selection for large, clean, disease-free seeds or other propagules for cross-pollinated (e.g. in maize; Soleri and Smith, 2002), self-pollinated (e.g. in barley; Ceccarelli *et al.*, 2000) and vegetatively propagated crops (e.g. in potato; Zimmerer, 1996). Selection with this goal is also conducted as part of MV seed multiplication (Simmonds and Smartt, 1999: 215). Plant breeders may also carry out this type of selection, for example by removing small seed, but they do this to decrease the contribution of σ_E^2 to σ_P^2, and so increase heritability with the goal of E.

Research on non-heritable phenotypic differences shows these can have important intra-generational effects in terms of ecology and agronomy. Even in species with high heritability for seed polymorphisms, environment may be an important determinant of seed size and shape, and seed polymorphism can be a significant determinant of differential survival via influence on survivorship and adult plant size (Baskin and Baskin, 2001: 208–214). In maize, for example, larger seed size was found to provide significant advantages in the early stages of plant growth (from germination until stem elongation) (Bockstaller and Girardin, 1994), and was correlated with better early vigour, greater leaf area throughout the life cycle and more rapid development from time of emergence to flowering (Pommel, 1990; Revilla *et al.*, 1999).

When the goal of selection is intra-generational phenotypic differentiation, the result may not be genetic response or evolution, especially for low-heritability traits in cross-pollinated crops. This hypothesis was supported by results of maize seed selection exercises with farmers in two communities in Oaxaca, Mexico. The exercises were done with maize ears post-harvest, which is the way these farmers and most others in Mexico select maize seed. Their selections resulted in high S

TABLE 14.2
Farmers' expectations for response to selection for their primary selection criterion in the major crop they grow

Country, crop, trait (n)	**Question A.** Farmers responding that response to intentional selection for 10 cycles > random selection for 10 cycles (IS_{10}>RS_{10})				**Question B**. For farmers responding IS_{10}>RS_{10} to Question A, those stating that response to intentional selection for 11 cycles > random selection for 10 cycles + intentional for 1 cycle (IS_{11}>RS_{10}+IS_1)			
	n	%		*P*	n	%		P
Mexico, maize, ear length (59)	23	39	*	0.000000	6	26	*	0.000000
Cuba, maize, ear length (29)	27	93		0.245614	12	44	*	0.000002
Syria, barley, plant height (21)	20	95		0.499999	11	55	*	0.000614
Nepal, rice, grain yield (40)	39	98		0.499999	17	44	*	0.000000
Mali, sorghum, grain yield (40)	35	88		0.057662	23	66	*	0.000078
Total (189)	144	76	*	0.000000	69	48	*	0.000000

One sided Fishers' exact test , of the null hypothesis that, similar to plant breeders, farmers would see intentional selection as achieving a greater response compared to random selection. Calculated using SISA (http://home.clara.net/sisa/). RS = random phenotypic selection by farmer, IS = intentional phenotypic selection by farmer.
Based on Soleri *et al.*, n.d.

Some farmers said large seed resulted in higher germination, larger seedlings, early vigour and higher yields, although most farmers attributed their preference for large seed to 'custom'.

It is still possible that simple mass selection for intra-generational phenotypic differences could result in *R* or *E* even if these are not farmer goals. As mentioned above, it is not clear what importance this had during domestication and subsequent diversification of crops, versus intentional selection for short-term change or long-term maintenance. For example, maize farmers in Uganda and the United Republic of Tanzania, like those in Mexico, were reported to select for large, clean kernels from large ears, apparently because they believed that these germinated well and produced high-yielding plants (Gibson *et al.*, 2005). Interestingly, this appeared to result in decreased resistance to maize streak virus, since resistant plants had smaller ears, and plants with large ears appeared to be non-resistant escapes.

As part of a comparative five-country study of FK and PBK (Soleri *et al.*, 2002, 2004), farmers were presented with a hypothetical scenario asking them to compare random with intentional selection for 10 cycles in a typical field, in populations with phenotypic variation for the trait they used as major selection criterion (Figure 14.6) (Table 14.2, question A). The null hypothesis was that farmers did not differ from plant breeders, i.e. that they would all consider intentional selection to be more effective than random selection for improving or at least maintaining this trait. The majority of responses corresponded to the null hypothesis of no difference between farmer and plant breeder expectations that intentional selection was more effective for increasing yield, 76.2 percent (144/189), although those who disagreed with that idea were sufficient to reject the hypothesis statistically ($P = 0.00000$). Disagreement was particularly frequent among maize farmers, probably due to recombination in that cross-pollinating crop.

These results indicate that farmers who believe there is an advantage of intentional over random selection, see their goal for phenotypic selection as either *S* or *R* or *E*. To discriminate between these possibilities, and with the same null hypothesis as

outlined above, those farmers responding to the first question that intentional selection resulted in greater yield, were asked to compare random selection for 10 cycles followed by one cycle of intentional selection, with 11 consecutive cycles of intentional selection. Results differed significantly from the null hypothesis (Table 14.2, question B). Among these farmers, only 23.2 percent (20/86) saw 11 years of intentional selection as superior. These results demonstrate that among those farmers favouring intentional selection, only a minority see it as providing cumulative multi-generational change (*E*), while the primary selection goal of the other farmers who saw an advantage to multi-generational intentional selection for low-heritability yield-related traits is either eliminating changes between generations (*R*) or a non-genetic advantage they believe is fully achieved within one year (*S*). The large number of farmers who do not consciously see an advantage to multi-generational intentional selection, but who, like other farmers, select for large seed from large, clean ears, may do so because of custom, as did the majority of farmers in the selection experiment described earlier.

14.6 CONCLUSIONS

Many elements of crop variety choice and plant selection in the Third World contrast substantially with industrial agricultural systems, including the growing environments, genetic resources and organization of the agricultural system. The urgency of understanding farmer selection will increase in the future with global climate changes, the continuing loss of genetic resources, the rapid spread of transgenic crop varieties, the development of a global system of IPR in crop genetic resources, the need to make agriculture more sustainable while feeding more people, and the movement to make formal plant breeding more relevant to farmers through PPB. Understanding farmers' choice and selection practices, their biological results, the knowledge and goals underlying them, and the similarities and differences with plant breeders provides a means for the two groups to work together more effectively. This understanding and collaboration is critical for supporting all of the important functions of SSTW agriculture, including long-term global food security.

For PPB, this means that farmers' goals for varietal choice and phenotypic selection need to be understood in the context of a system that integrates production, consumption, improvement, multiplication and conservation. The biological result of phenotypic selection needs to be evaluated in terms of its possible ecological effects (via *S*), as well as in terms of *R* and *E*. Additionally, farmers' theoretical knowledge of choice and selection, not just their criteria, need to be understood by plant breeders to fully realize the potential benefits of collaboration. The value of this research will be judged by its effectiveness in improving the efficiency and outcomes of collaborative breeding by scientists and farmers, and improvement in the well-being of those farmers and their communities.

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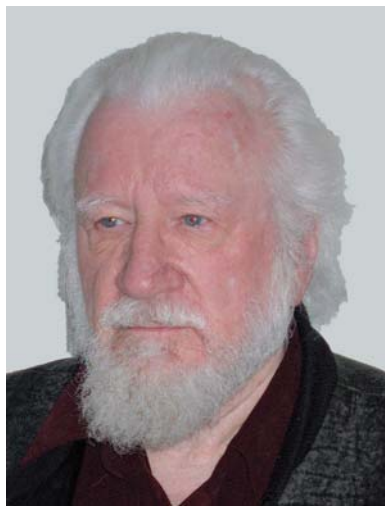
CHAPTER 15

Breeding for quantitative variables Part 2: Breeding for durable resistance to crop pests and diseases

Raoul A. Robinson



15.1 INTRODUCTION

There are three categories of plant breeder. First is the professional, who is a highly trained scientist with a profound knowledge of modern genetics and related subjects. Second is the amateur, who uses simple selection techniques to produce new varieties. Third is the subconscious selector who unwittingly changes plants by artificial selection. It is these subconscious selectors who were responsible for all the original domestication of plants, and for much of the modern artificial selection in Third World countries.

There were no professional plant breeders before 1900, and all plant breeding was done by amateurs and subconscious selectors. The classic example of an amateur was a farmer called Rimpau who, in 1866, started selecting the best rye plants in his crop for use as seed. He did this with each successive crop and, after twenty years, he had a greatly superior rye known as 'Schlanstedt', which quickly became popular in much of Europe. In this chapter, participatory plant breeding is taken to mean cooperation between professional and amateur plant breeders.

During the twentieth century, there were major changes. Between 1900 and 1905, three seminal discoveries were made. These were the recognition of Mendel's laws of inheritance, Johannsen's discovery of pure lines, and Biffin's discovery of single-gene resistances. All subsequent plant breeding was done by scientific professionals, who were totally captivated by these new discoveries. Amateur plant breeders disappeared almost entirely. More recently, the techniques of genetic engineering have become popular, but these, of necessity, involve single-gene resistances, and they usually require professional scientists.

The switch from amateur to professional breeding occurred because there are two kinds of breeding, depending on whether the breeding is for multiple-gene or single-gene characters. Rimpau was working with multiple-gene characters, and he obtained small, quantitative improvements with each generation of selection. However, when it came to resistance to crop pests and diseases, the professional breeders were working with single-gene resistances, because they had a choice and they chose this method as it gives a quick response. This single-gene breeding is usually too complex and too difficult for amateur breeders, and this was why they disappeared during the twentieth century.

Today, the pendulum is swinging back again, and there is a new appreciation of the value of both amateur breeders and multiple-gene resistances. Participatory plant breeding, using multiple-gene resistances, now provides an admirable opportunity for cooperation between amateurs and professionals.

15.1.1 Crop parasites

In this chapter, a crop parasite is defined as any organism that spends a major part of its life cycle inhabiting one host individual, and obtaining nutrients from that host. The host, of course, is a crop plant. A parasite may be an insect, mite, nematode, parasitic angiosperm (e.g. broomrape, witchweed, dodder), or any of the various categories of plant pathogen, such as fungi, bacteria and viruses. However, weeds are not parasites: they are competitors, and they are not part of this discussion.

This chapter is addressed to both participating, amateur plant breeders, who may find *Return to Resistance* (Robinson, 1996) helpful, and to cooperating professional breeders who may find *Self-Organising Agro-Ecosystems* (Robinson, 2004) useful.

15.1.2 Plant breeding

For the whole of the twentieth century, scientific plant breeding has had four main objectives. These were improvements in (i) yield; (ii) quality of crop product; (iii) agronomic suitability; and (iv) resistance to parasites. This scientific breeding has been remarkably successful in the first three of these objectives, but much less successful with breeding for resistance. The basic reason for this has been that resistance kept breaking down because of new strains of the parasite. There was then an apparently endless repetition of resistance failures and a 'boom and bust' cycle of cultivar production.

Vanderplank (1963, 1968) first made a clear distinction between single-gene and multiple-gene resistances to crop parasites. He called single-gene resistance 'vertical resistance', and it is normally qualitative in that it provides complete protection or none at all, with no intermediates. He called multiple-gene (polygenic) resistance 'horizontal resistance', and it is quantitative in that it can provide every degree of protection from a minimum to a maximum.

15.1.3 Stability and instability

Any protection mechanism against a crop parasite may be described as unstable or stable. An unstable resistance is *within* the capacity for micro-evolutionary change of the parasite. This means that the parasite is able to produce a new strain that is unaffected by that protection, which is then said to have 'broken down' (strictly speaking, the protection is unaltered, and it is the parasite that has changed). Many synthetic insecticides and fungicides provide unstable protection, and they sooner or later break down in the face of new strains of the parasite. Single-gene, vertical resistances are almost always unstable, and they too break down as new races, strains, biotypes or pathotypes of the parasite emerge.

Horizontal resistances provide stable protection. That is, they are *beyond* the capacity for micro-evolutionary change of the parasite, which is consequently unable to produce a new strain that is unaffected by that resistance. Other protection mechanisms can also be stable. Examples of stable insecticides include natural pyrethrins, rotenone, and a film of oil on water to control mosquito larvae. Examples of stable fungicides include both copper and dithiocarbamate formulations.

15.1.4 Vertical resistance

Vertical resistance has several remarkable advantages. First, it provides complete protection against a parasite. There are a few examples of incomplete vertical resistance (see below) but not enough to invalidate this rule. Second, it usually has a very wide climatic range and can be employed across broad geographical regions. Third, being controlled by single genes, it is amenable to gene-transfer and back-crossing techniques. These techniques are so elegant, and so beautiful, that they were greatly favoured by professional breeders during the twentieth century.

However, vertical resistance does have some grave disadvantages. First, as already mentioned, it is unstable. It is liable to break down when faced by new strains of the parasite. The speed of this breakdown can vary greatly. The fastest occurs in the first growing season, and this happened with *Puccinia polysora* of maize in tropical Africa (see below), and in Late potato blight (*Phytophthora infestans*) in the Toluca Valley of Mexico, which is the centre of origin of this fungus. The slowest breakdowns take so long to occur that the vertical resistance is effectively durable (see

below), but these are too rare to be a general breeding tool.

A second disadvantage of vertical resistance is that it is responsible for the vertifolia effect, which is the gradual loss of horizontal resistance during breeding for vertical resistance, and which is described in more detail below. A third disadvantage is that vertical resistances occur only against some species of parasite. Consequently, it is not possible to use vertical resistance for all the locally important parasites.

In general, vertical resistance is not recommended for participatory plant breeding. This is mainly because the failure of a wonderful new cultivar, which has taken years of devoted work to produce, by both professional and amateur breeders, is quite frankly heart-breaking. Nothing can be expected to discourage amateur breeders more than this. An essential aspect of participatory plant breeding is that we maintain the confidence of the participating amateurs. Consequently, participating professional breeders should be very cautious about recommending the use of vertical resistance, or even the combined use of vertical and horizontal resistance, in participatory plant breeding.

15.1.5 Horizontal resistance

Being a quantitative variable, horizontal resistance can be expressed at any level between a minimum and a maximum. In the absence of crop protection chemicals, the minimum level of horizontal resistance usually results in a total loss of crop from the parasites. And the maximum level of horizontal resistance usually results in negligible loss of crop. However, the maximum level of horizontal resistance never provides as complete a protection as vertical resistance. Even with the highest level of horizontal resistance, there is always some slight parasitism.

The main advantage of horizontal resistance is that it is durable, and that it is possible to breed for increased levels of many different quantitative variables simultaneously. Participatory plant breeders can accordingly aim at high levels of horizontal resistance to all locally important parasites. This will achieve crop husbandry that is effectively free of all parasite damage, and one that is free of pesticides as well. And these freedoms will be permanent. However, it must be emphasized that this is the objective. Such an objective may prove impossible to achieve in practice, at least in some crops, and in some areas. But, even if this objective is unattainable, there will be at least some improvement over current farming practices in terms of increased yields and decreased damage from parasites.

15.1.6 Two kinds of plant breeding

Clearly, the key difference in breeding crop plants for temporary and durable resistances is the difference between breeding for single-gene and multiple-gene characters.

Breeding for single-gene characters requires both pedigree breeding and backcrossing, or the very modern techniques of marker assisted selection and genetic engineering. Anyone using these techniques for acquiring resistance should assume that the resulting resistance will be unstable, and that it will have a very high probability of breaking down sooner or later.

Breeding for multiple-gene characters, such as horizontal resistance, requires an entirely different breeding technique, called recurrent mass selection, which is discussed below.

15.2 WHY WAS TEMPORARY RESISTANCE SO POPULAR?

During most of the twentieth century, vertical resistance was consistently the resist-

ance of choice, and horizontal resistance was almost entirely ignored. This is a historical fact, and it is so important that we must examine its causes in some detail.

The effectiveness of horizontal resistance is influenced by many quantitative variables. Many of these variables are difficult to observe or measure, and there is a powerful tendency to neglect them. Twentieth-century plant breeders did neglect them, and this is why they also neglected horizontal resistance. They concentrated on vertical resistance because it is complete. This completeness was very attractive. It meant that the parasite control was total. However, the ephemeral nature of the resistance was usually revealed only much later.

Today, if we are to breed crops successfully for resistance that is durable, we must understand these misleading, quantitative variables that disguise the effectiveness of horizontal resistance. Within the framework of participatory plant breeding, a primary function of the participating specialists must be to ensure that the participating farmers are not deceived by these misleading variables.

15.3 MISLEADING VARIABLES

In principle, breeding for horizontal resistance is very simple and very easy and for this reason it is ideal for participatory plant breeding. But there are a number of factors that can be horribly misleading and which, unless understood, can lead to totally false observations. These false observations often disguise horizontal resistance so effectively that little genetic advance can be seen.

It was these sources of error that led to vertical resistance being the preferred resistance mode among plant breeders for the whole of the twentieth century. Being qualitative, vertical resistance is easily seen, but its unstable nature is not apparent. Being quantitative, in contrast, horizontal resistance is easily obscured, its durability not recognized, and its value consistently underestimated. A clear comprehension of these misleading variables, these sources of error, is consequently essential for anyone wishing to breed crops for durable resistance. The twelve most important of them are considered below. They are summarized in Table 15.1.

15.3.1 Parasite interference

The only way to measure the level of horizontal resistance is by the level of parasitism. Parasite interference can make such assessments wildly inaccurate, and the level of parasitism is then a thoroughly misleading indication of the level of horizontal resistance. Parasite interference occurs because the parasites are mobile, and they can move from plot to plot, or from plant to plant. The importance of this phenomenon was first discovered by Vanderplank (1963).

Parasite interference can be seen with field trials in which the parasites can move from one plot to another. The results of parasite interference can be extraordinary, and may lead to errors of several hundred-fold (James *et al.*, 1973). In these circumstances, the effects of horizontal resistance can be totally obscured. Parasite interference is at its worst in the very small screening plots produced by the technique of 'ear-to-row' selection (or family selection), often used during the breeding of self-pollinating small-grain cereals and pulses. The parasite interference can be so intense that plants with a functioning vertical resistance can appear diseased, because of millions of hypersensitive flecks produced by the failed infections of non-matching strains of the parasite. Imagine the level of parasitism, therefore, if those infections are produced

TABLE 15.1
Summary of misleading variables

Misleading Variable	Problem	Solution
Parasite interference	Hides high levels of horizontal resistance	Use relative measurements only
Epidemiological competence	Resistance requirements vary between agro-ecosystems	Use on-site screening
Environmental erosion	An apparent loss of horizontal resistance due to re-location in an area of higher epidemiological competence	Each agro-ecosystem should have its own breeding programmes
Vertifolia effect	The loss of horizontal resistance when breeding crops for vertical resistance, or under the protection of crop protection chemicals	Inactivate all vertical resistances, and avoid any use of crop protection chemicals
Parasite erosion	An apparent loss of horizontal resistance due to changes in the parasite population	Screen in an area of high parasite density
False erosion	An apparent loss of horizontal resistance due to sloppy or negligent assessments	Be more careful
Biological anarchy	An increased epidemiological competence in the parasite due to the loss or debilitation of biological control agents	This phenomenon will disappear after a few seasons of freedom from pesticides, as the biological controls are restored by the use of horizontal resistance
Population immunity	Makes field assessments preferable	Avoid laboratory assessments of resistance
Chance escape	Provides a false indication of resistance	Inoculate the screening population, or use grid screening
Quantitative vertical resistance	Looks like horizontal resistance but is not	Use parents that lack vertical resistance genes, or use the one-pathotype technique (see below).
Durable vertical resistance	This rare phenomenon provides a false hope for breeders of single-gene resistances	Do not rely on this remote possibility
Sub-optimization	Do not breed for a single resistance mechanism, or resistance to single species of parasite	Use the holistic approach (see below)

by a matching strain, against which the vertical resistance does not operate.

Parasite interference can also occur among individual host plants. Consider a screening population in which there are wide differences in horizontal resistance between individual plants. A quantitatively resistant plant may be surrounded by susceptible plants. The overcrowded parasites will then move onto that resistant plant, making it look far more susceptible than it really is. Once again, the magnitude of these errors can be great. In fact, if that resistant plant were growing in a farmer's field, as a pure line or clone, there would be no parasite interference, and its resistance might then be entirely adequate to provide complete control of the parasite.

The misleading effects of parasite interference can be avoided during breeding for horizontal resistance by using *relative* measurements during screening. That is, the *least parasitized* individuals are selected regardless of how severely parasitized they might be. In the early breeding cycles, these least-parasitized individuals may well look so awful that the breeder could be forgiven for concluding that there is no point in continuing. But that would be a mistake. However awful they may look, they are the least susceptible individuals of an early breeding cycle and, as such, they should become the parents of the next breeding cycle.

Remember also that we are breeding for comprehensive horizontal resistance

to all the locally important parasites. This requires the one quality of 'good health'. The least parasitized individuals will be fairly susceptible to many different species of parasite. Their quality of 'good health' will be low. But it will be higher than all those other, less healthy individuals in the screening population, many of which may have disappeared entirely.

A final comment about parasite interference concerns the movement of parasites from one farm, or one district, to another. Many organic farmers are able to cultivate fairly susceptible cultivars successfully because their neighbours are using crop protection chemicals. The district interference is then minimal. But were all the farmers in that district to eschew crop protection chemicals, the parasite populations would be so large, and the district interference so great, that the use of those cultivars for organic agriculture might prove impossible. The other side of this coin is that progress in breeding for horizontal resistance will lead to reductions in district interference, which, in turn, will enhance the effects of that horizontal resistance.

15.3.2 Epidemiological Competence

Epidemiological competence refers to the ability of a crop parasite to cause an epidemic (or infestation). It is another biological variable that can be expressed at any level between a minimum and a maximum.

Consider a wild ecosystem, which might, perhaps, extend up a mountainside. The epidemiological competence of a plant parasite might change along a gradient from low to high moisture, or temperature, or whatever factor is governing that epidemiological competence within the ecosystem. The various host ecotypes along that gradient will have corresponding levels of horizontal resistance. Where the epidemiological competence is low, the horizontal resistance will also be low, because there is little selection pressure for resistance. But where the epidemiological competence is high, the level of horizontal resistance will also be high, because there is strong selection pressure for resistance.

When breeding for horizontal resistance, this variation in epidemiological competence is important in two ways. First, we must use 'on-site screening'. This means that the screening for horizontal resistance must be conducted: (i) in the locality of future cultivation; (ii) in the time of year of future cultivation; (iii) in the field (i.e. not in the laboratory or greenhouse); and (iv) according the farming system (e.g. organic or conventional, irrigated or rainfed) of future cultivation. On-site screening is particularly well suited to participatory plant breeding.

Second, each distinct agro-ecosystem will require its own horizontal resistance breeding programme for each of its crop species. This programme must be aimed at the levels of epidemiological competence of the locally important parasites. In practice, this is not difficult. Agro-ecosystems are usually quite large, and the necessary levels of horizontal resistance will be discovered by practical farming experience, spread over time, as the breeding programmes produce more and more new cultivars, with higher and higher levels of comprehensive horizontal resistance.

15.3.3 Environmental erosion of horizontal resistance

If a cultivar has adequate horizontal resistance in an agro-ecosystem in which the parasite has a relatively low epidemiological competence, and that cultivar is taken to a different agro-ecosystem, where the parasite has a high epidemiological competence, the

resistance will appear to have decreased, possibly disastrously. This is known as environmental erosion of horizontal resistance. Strictly speaking, of course, the resistance is unchanged and it is the epidemiological competence of the parasite that is different. But the level of parasitism increases and this can be alarming if the cause is not understood.

It is because of these differences in epidemiological competence between agro-ecosystems that we speak of 'locally important parasites'. We are breeding agro-ecotypes (i.e. cultivars) for our own agro-ecosystem. These agro-ecotypes may have too much, or too little, resistance in other agro-ecosystems, where the epidemiological competences are different. But these other agro-ecosystems are not our concern. Equally, other people's agro-ecotypes will very likely prove inferior in our own agro-ecosystem. Professional breeders often speak of site-specific plant breeding in which the breeding targets are aimed at a particular site with special requirements. On-site selection fits in well with this concept.

The environmental erosion of horizontal resistance seems like a breakdown of that resistance but, clearly, it is not.

15.3.4 The vertifolia effect

The vertifolia effect was first recognised by Vanderplank (1963) and it is an erosion of horizontal resistance resulting from genetic changes in the host species which occur during breeding for vertical resistance. These changes can also occur during any breeding that is conducted under the protection of crop protection chemicals.

This erosion occurs because horizontal resistance can be measured only in terms of the level of parasitism. If the level of parasitism is totally obscured by pesticides, or by a functioning vertical resistance, the level of horizontal resistance cannot be observed or assessed. Because individuals with the highest levels of horizontal resistance are always a minority in a screening population, less-resistant individuals are more likely to be selected on the basis of their other attributes.

After many generations of crop breeding conducted under the protection of pesticides or vertical resistance, or both, the levels of horizontal resistance in modern cultivars are usually low. This erosion of horizontal resistance has continued for about a century in many species of crop. It has been particularly serious in potatoes, tomatoes and cotton, but there are few species in which it has not occurred. It is also the reason why breeding for horizontal resistance is now so important.

The lesson of the vertifolia effect is that we must never protect our screening populations with crop protection chemicals. However, there is one exception to this rule. In the early screening generations, even the least parasitized individuals may be so severely damaged that their very survival is threatened. In such a case it is permissible to use crop protection chemicals as a last resort to preserve the parents of the next generation. Equally, we must never use vertical resistances in our screening populations. Methods of avoiding this are described below.

People who call themselves 'seed savers' know that century-old cultivars, often called 'heirloom varieties', usually have higher levels of durable resistance than modern cultivars. This difference is a measure of the overall vertifolia effect that has occurred during the twentieth century.

15.3.5 Parasite-erosion of horizontal resistance

Occasionally there can be changes in the parasite population that lead to an

increased epidemiological competence. This can happen, for example, with the *Fusarium* and *Verticillium* wilt fungi. The frequency of the highly pathogenic forms of these soil-borne pathogens might be quite low in the area of field screening. However, with repeated use of one field for the screening work, the frequency of pathogenic forms will increase, more or less in step with the increases in horizontal resistance in the host. The overall effect is then an appearance of no progress whatever, with a very real possibility of the breeding programme being abandoned.

This is a somewhat rare phenomenon and the participatory breeder is unlikely to be faced with it very often. However, it is as well to be aware of the possibility, just in case of apparently discouraging progress. As a general rule, however, the parasitic ability of most crop parasites, particularly the obligate parasites, is fixed, and parasite-erosion is rare.

15.3.6 False erosion of horizontal resistance

There can be false erosion of horizontal resistance due to sloppy techniques, or just plain carelessness. The classic example of this occurred with a virus disease of sugar cane called mosaic. This disease can be devastating when susceptible cultivars are being cultivated. However, in most areas, the disease was controlled so completely with horizontal resistance that it tended to be forgotten. New cultivars that were released to farmers were susceptible because they had been inadequately tested, or not tested at all, for resistance to this virus, and there would then be a flare up of the disease. It was not uncommon for this to be blamed on a breakdown of resistance when, of course, it was nothing of the kind.

15.3.7 Biological anarchy

Biological anarchy is the converse of biological control. It means that the various agents of biological control have been depleted or destroyed by crop protection chemicals, particularly when there has been pesticide overload. These agents might be hyper-parasites, predators, microbiological competitors, toxin-producing micro-organisms, or organisms that stimulate resistance responses in the host. The importance of biological anarchy is revealed by the success of integrated pest management, generally known as IPM. This method involves careful monitoring of the crop parasites in order to reduce the use of crop protection chemicals to the absolute minimum necessary for control. A gradual increase in biological control then occurs. When the biological controls are restored, a greatly reduced rate of crop protection chemical application can be maintained.

The importance of biological anarchy during breeding for horizontal resistance is that, in the absence of biological controls, many crop parasites will behave with a savagery that would be impossible if the biological controls were functioning. This means that assessment of the level of horizontal resistance can be difficult. Screening for horizontal resistance should be conducted in an area where there is no biological anarchy. But, given the widespread use of crop protection chemicals, particularly in the industrial nations, it is often impossible to find such an area. The screening population then appears to have so little resistance that grave doubts develop concerning the wisdom of this approach. However, the problem of biological anarchy is less acute in developing countries, where the use of crop protection chemicals is much less intense.

It should be added that the best way to restore biological controls is to use horizontal resistance. And the best way to enhance the effects of horizontal resistance is to restore biological controls. The two effects are mutually reinforcing. The practical effect of this is that a new cultivar with apparently inadequate horizontal resistance may well prove to have adequate horizontal resistance, once the biological controls are restored. This restoration may require several seasons of cultivation without pesticides but, once complete, the effects can be dramatic.

It should also be added that the agents for biological control often depend on a small population of the parasite in order to maintain their own populations. A low level of parasitism is often desirable for this reason, provided that it has no deleterious effect on the yield or quality of the crop product. However, purchasers of organic food often like to see minor parasite damage as evidence for freedom from crop protection chemicals.

15.3.8 Population immunity

Population immunity means that a host population is effectively immune, even though the individuals that make up that population are less than immune. This is because population growth, unlike an individual's growth, can be positive or negative. Positive population growth means that there are more births than deaths, and the population is increasing. Negative population growth means that there are more deaths than births, and the population is decreasing. The dividing line occurs when the births and deaths are equal, and the population growth is then zero.

Now consider a crop parasite. In order to cause an epidemic (or infestation), the parasite population growth must be positive. Indeed, it must be strongly positive if it is to become a serious crop pest or disease. Each individual parasite must spawn more than one new individual before it dies. Now suppose that the combination of horizontal resistance and biological controls is such that, on average, each parasite individual spawns less than one new individual. The parasite population growth is now negative, even though the host individuals are not immune. This situation is population immunity, and it is important in horizontal resistance breeding in three quite different ways.

First, population immunity means that we do not need to breed for the maximum levels of horizontal resistance. We need to breed for enough horizontal resistance to cause population immunity, and no more. This level is discovered from practical farming experience.

Second, although vertical resistance can be assessed on detached leaves in a test tube, or leaf disks in a Petri dish, or even entire plants in a growth chamber, horizontal resistance should not be measured in this way. This is because these laboratory methods cannot possibly represent the effects of biological controls or population immunity. The levels of horizontal resistance are best determined in the field and, if at all possible, under conditions of restored biological controls. Once again, the best determinations will be the result of practical farming experience.

Third, all measurements and descriptions of horizontal resistance must be relative. There can be no absolute measurements. We can describe a new cultivar "A" as being more resistant to a particular parasite than cultivar "B", but less resistant than cultivar "C". But we cannot have an absolute scale of measurement comparable to the Celsius scale of temperatures. This is because these

biological variables are too imprecise to define accurately. However, this does not make horizontal resistance any less useful in farmers' fields.

15.3.9 Chance escape

The distribution of parasites in a screening population is often uneven. This is called a 'patchy distribution' and it is most common with soil-borne parasites and gregarious insect pests. There may then be some host individuals that have no parasites at all, and they give the impression of being highly resistant. They are known as 'chance escapes' and, obviously, they should not be selected as parents of the next screening generation because they could be very susceptible. But the problem is how do we recognise them, and how do we avoid them? There are a number of techniques that increase the accuracy of the screening, depending on the nature of the parasite. It is in this area that the professionals will be most useful to participatory amateur breeders.

If the parasite is soil-borne, such as a root nematode, or a fungal or bacterial wilt organism, it is a good idea to pre-germinate the seedlings in flats or peat pots for later transplanting in the field. These flats or pots can then be inoculated with the parasites in question, and the very process of transplanting will ensure an even distribution of the parasite.

If the parasite is a gregarious insect, in which all individuals tend to congregate on one host plant, they can often be redistributed on a daily basis by disturbing them. This can be particularly important with virus vectors.

If the parasite is seed-borne, it is usually feasible to inoculate the seed before sowing. The details of the techniques for doing this vary considerably with different kinds of parasite and, with participatory plant breeding, this will be the responsibility of the professionals.

If the parasite is wind-borne, such as most fungal spores, or a flying insect, a previously prepared population of the parasite can be released, blown or water-sprayed on to the screening population. Once again, the details of the techniques vary and will be handled by the professionals.

With some parasites, inoculation is not feasible for technical reasons. An alternative technique is then to ignore those parts of the screening population that are free of the parasite in question. Or any individual plant that is entirely free of the parasite in question can be ignored. This procedure runs the risk of discarding some highly resistant potential parents, but this wastage is preferable to the risk of selecting highly susceptible escapes as parents of the next generation.

Finally, there may be parasite gradients within the screening population in which the intensity of parasitism changes gradually from low to high from one part of the host population to another. This effect can be eliminated by dividing the screening population into a grid of suitably sized squares. Only the least parasitized individual is selected within each square, regardless of the level of that parasitism when compared with other squares.

15.3.10 Quantitative vertical resistance

Occasionally, vertical resistance can be quantitative and it is then easily confused with horizontal resistance. It occurs, for example, with Hessian fly (*Mayetiola destructor*) of wheat (Dent, 1998). Fortunately, this situation is rare and need not worry the breeders of most crops. However, if it does occur, quantitative vertical resistance must obviously be avoided or inactivated during

breeding for horizontal resistance. This can be done either by using only parents that are known to possess no genes for quantitative vertical resistance. Alternatively, the 'one-pathotype technique' (described below) may be employed.

15.3.11 Durable vertical resistance

Very occasionally, single-gene vertical resistances may be durable. This happens, for example, with cabbage yellows (*Fusarium oxysporum* f.sp. *conglutinans*) in the United States of America, potato wart disease (*Synchytrium endobioticum*) in Britain, and wheat stem rust (*Puccinia graminis*) in Canada (Vanderplank, 1978). The durability is usually due to local circumstances that would not occur in the wild pathosystem of the host and parasite progenitors. However, durable vertical resistance can be very useful, and there is no reason why it should not be exploited. But durable vertical resistance is so rare that it should not be aimed at, nor depended on, in most breeding programmes. It led many plant breeders astray in the twentieth century, as they continued to hope, over-optimistically, that their single-gene resistances would also prove to be durable.

A technique that has had some success is the 'pyramiding' of single-gene resistances. This involves putting as many different vertical resistance genes as possible into one plant in a so-called 'pyramid', and this can prolong the life of vertical resistance, particularly if genes from different species of wild plants are combined. However, this is resistance breeding at its most difficult and is of doubtful value for participatory plant breeders. If it is employed, it will require professional plant breeding at a plant breeding station combined with selection in farmers' fields.

15.3.12 Sub-optimization

Sub-optimization means emphasizing some subsystems at the expense of others, usually by working at too low a systems level. There are many levels of subsystem in biological systems and sub-optimization can lead to two kinds of error. First, by working at too low a systems level, other subsystems may be overlooked. Second, emergent properties, which can be observed only at their own systems levels, may also be overlooked.

An obvious example of sub-optimization in biology occurs with the 'schooling' of fish. This schooling is an emergent property in which a population of fish swim as one individual. A scientist studying only one fish, or even a pair of mating fish, in an aquarium, cannot possibly observe this emergent property of schooling. In order to study schooling, the scientist must work at the higher systems level of the population.

During the twentieth century, plant breeding for resistance to parasites suffered considerably from sub-optimization. Research was conducted at the systems level of the individual host or parasite, or even the individual resistance mechanism. It should also have been conducted at the level of the two interacting populations of host and parasite, which is a systems level now called the *pathosystem*. A pathosystem is a subsystem of an ecosystem and, when host resistance is studied at this level, very different pictures of both vertical resistance and horizontal resistance emerge. This is because of previously unobserved emergent properties.

We now recognize the significance of two different kinds of infection. Infection is defined *as the contact made by one parasite individual with one host individual for purposes of parasitism.* With allo-infection, the parasite has to travel to its host, having

originated somewhere else. This is analogous to cross-pollination, or allogamy. With auto-infection, the parasite is born, hatched or spawned on the host that it is infecting. This is analogous to self-pollination, or autogamy. We also recognize that, in a wild pathosystem, vertical resistance can control allo-infection only, and auto-infection can be controlled only by horizontal resistance (although horizontal resistance can also control allo-infection).

The control of allo-infection by vertical resistance apparently operates as a system of locking, with each host having a biochemical lock, consisting of several resistance genes, and each parasite having a biochemical key consisting of several parasitism genes. If the parasite key does not fit the lock of the host it is allo-infecting, the infection fails, while if the key does fit the lock the infection succeeds. Such a system ensures that the frequency of matching allo-infection is low, and this stabilizes the population explosion of the parasite. This stabilization is an emergent property that can be seen only at the level of the system of locking, the level of the pathosystem.

However, if every door in the town has the same lock, and every householder has the same key, which fits every lock, the system of locking is ruined by uniformity. And this is exactly what we have done in agriculture, with our use of a single vertical resistance in a uniform pure line, clone or hybrid cultivar that might be grown over a huge area as a homogeneous population. This was sub-optimization at its worst.

Auto-infection can be controlled only by horizontal resistance because auto-infection can commence only after there has been a matching allo-infection. Many crop parasites reproduce asexually to produce a clone. All the individuals within that clone have the same key which matches the lock of the host and, consequently, all auto-infection is matching infection. Even parasites that reproduce sexually, such as many insects, will soon reach homozygosity of the matching biotype. However, vertical resistances against insects are rather rare, and this may explain why there has been so little crop breeding for resistance to insects pests.

These functions of the two kinds of resistance are emergent properties that were completely unknown until recently, and, being unknown, they were inevitably ignored by crop scientists during the twentieth century.

Another obvious example of sub-optimization occurs when breeding for a single resistance mechanism, such as hairy leaves that repel an insect pest. Horizontal resistance to insects usually consists of many obscure mechanisms, all of which may vary quantitatively, and which collectively reduce the rate of population growth of that pest.

The converse of sub-optimization is known as the holistic approach, which leads to local optimization of all variables. When breeding for horizontal resistance, therefore, we must not sub-optimize. We must work at the systems level of the agro-ecosystem. Within this agro-ecosystem, we must use population breeding to produce adequate and durable resistance to all locally important species of parasite, by exerting selection pressure for the one characteristic of 'good health'. Susceptibility to only one important species of parasite will result in an inferior cultivar, and this would constitute a clear case of sub-optimization. In addition to 'good health', new cultivars should have good levels of all the other variable attributes necessary to a productive agriculture. This approach does not necessitate participatory plant breeding, and some professional

breeders may choose to work on their own in a scientific institution. However, this approach does depend on high numbers of plants being screened, and many amateur breeders, working cooperatively with a professional, can lead to both greatly increased numbers of plants screened, and greatly increased attention applied to each plant screened.

15.4 BREEDING CROPS FOR DURABLE (HORIZONTAL) RESISTANCE

In this section there are inevitable generalizations that do not apply to all crop species. For example, comments about open-pollinated crops may not apply to self-pollinated crops, or comments about annual crops may not apply to perennial crops. When planning a breeding programme, therefore, readers should highlight only those aspects of these descriptions that apply to their crop species of choice.

15.4.1 Maize in tropical Africa

The best way to breed for horizontal resistance is to imitate the behaviour of open-pollinated maize (*Zea mays*) in tropical Africa, following the introduction of the re-encounter disease called Tropical rust (*Puccinia polysora*). A re-encounter parasite is one in which the host was separated from its parasite and taken to another part of the world. At a later date, the parasite is also taken to that new area where it re-encounters its host, which has lost resistance in the meanwhile. Conversely, a new encounter parasite is one which evolved separately from its host, on a botanical relative. Later the two are brought together in a new encounter. An old encounter parasite is one in which the host and parasite have never been separated, even though both may have been moved to new areas (Buddenhagen, 1987).

This crop is called corn in North America but it is called maize in all other countries, and in all other languages. It was taken by the Spanish from the New World to the Iberian peninsula some five centuries ago and tropical rust was either left behind or it failed to survive outside the tropics. The Portuguese then took rust-free maize to Africa and all points east, where it was cultivated for more than four centuries in the absence of tropical rust. This negative selection pressure led to the level of horizontal resistance to tropical rust declining to its minimum natural level. In technical terms, this is the Hardy-Weinberg equilibrium. In theory, it should be possible to breed experimentally for absolute susceptibility, but this is a somewhat academic point.

With the development of trans-Atlantic air transport in the 1940s, tropical rust was accidentally taken from the new world to the old. Devastating epidemics developed in the low altitude, equatorial tropics. In East Africa, a classic breeding programme for vertical resistance was initiated. Genes for resistance had to be imported from tropical America because none could be found in the local maize populations (Storey *et al*., 1958). Unfortunately, these vertical resistances broke down so quickly that none lasted long enough to be released to farmers.

However, the appearance of this re-encounter disease exerted positive selection pressure for horizontal resistance in the farmers' open-pollinated crops. After about a dozen maize generations, the levels of disease had declined from 'total loss of crop' to 'negligible loss of crop'. The horizontal resistance had increased from its minimum level to its maximum level. With two crops each year, this transformation occurred in about six years, and it had happened without any help from plant

breeders or plant pathologists. It is common knowledge that this horizontal resistance has now endured for half a century without any suggestion of breaking down to new strains of the parasite.

15.4.2 The inactivation of vertical resistance

It is impossible to breed for horizontal resistance if the screening population is protected by functioning vertical resistances. In other words, it is possible to breed for horizontal resistance only *after* the vertical resistances have been matched.

There are several methods of ensuring that no vertical resistances are functioning during screening for horizontal resistance. The first method is to use only parents that possess no genes for vertical resistance. This is usually possible only in crops species that have had foreign vertical resistance genes inserted into them. For example, the vertical resistance genes to Late potato blight (*Phytophthora infestans*) were inserted into cultivated potatoes from the wild *Solanum demissum*.

In this context, it is important to note that vertical resistance genes occur only in seasonal tissues in which there is a discontinuous pathosystem. That is, in each new season, each host individual is free of the parasite and must be newly allo-infected. This situation is seen in the seasonal tissues of annual plants, or the leaves and fruits of deciduous trees and shrubs. Vertical resistances can also occur in the seasonal, aerial tissues of perennial crops such as hops and potatoes. But vertical resistances are not found in perennial crops such as sugar cane, cassava and sweet potato, or in evergreen tree crops such as olives, citrus, tea and cocoa. Apparent exceptions to this rule occur in Coffee leaf rust caused by *Hemileia vastatrix*, and South American leaf blight of rubber, caused by *Microcyclus ulei*. However, coffee is functionally deciduous in the dry season with respect to rusted leaves only, and rubber is a deciduous species in spite of growing in the Amazon valley, which is permanently warm and wet.

The second method involves inactivation of any vertical resistance genes that may be present in the screening population. This is necessary in crops, such as wheat, in which it is extremely difficult, if not impossible, to find parents with no vertical resistance genes. This inactivation can be achieved with the 'one-pathotype technique'. The first step is to designate a single vertical pathotype of the parasite in question. Potential parents are then screened for susceptibility to this designated vertical pathotype. Only those lines that are susceptible to the designated vertical pathotype can become original parents of the screening population. All recombinations of the vertical resistance genes that occur in all subsequent breeding generations will then be matched by the designated vertical pathotype, which is used to inoculate each screening population. Amateur breeders will have difficulties with this somewhat complex but essential procedure, and in a participatory breeding programme it should be the responsibility of the breeder or pathologist. The details of the technique have been described elsewhere (Robinson, 1996, 2004).

A third possibility is to rely on natural matching of any vertical resistances that may be present in the screening population. Any individual that has no parasitism is inspected for the presence of the necrotic spots that are typical of a vertical resistance reaction, and affected individuals are discarded. However, this method can be very wasteful of breeding material if there are many such plants.

It is not clear whether the maize in tropical Africa lacked vertical resistance genes, or that it had such genes but their resistances were matched so quickly that they went unobserved. In either event, the positive selection pressure for horizontal resistance was not hindered by functioning vertical resistance.

15.4.3 Genetic resources

It is now a plant breeding shibboleth that breeding for resistance requires a 'good source' of resistance before the breeding can even begin. This is true when breeding for vertical resistance, because it is essential to have at least one gene for resistance. But it is *not* true when breeding for horizontal resistance. This point is so important that it merits careful explanation.

Consider a heterogeneous screening population in which every individual is different from every other individual, but in which all the plants are susceptible. Each plant possesses about 10 percent of the total polygenes that contribute to the horizontal resistances to each of the various locally important parasites. The host population as a whole is thus very susceptible. However, we may assume that each host individual possesses a different 10 percent of those total polygenes. Provided that there is a reasonably wide genetic base, this will mean that all the polygenes are present in the population, but their frequency is too low for much resistance to be expressed in any of the individuals. The objective of the breeding is to increase these resistance gene frequencies.

The most resistant plants are selected and they become the parents of the next screening generation. Now the most resistant individuals will possess perhaps 20 percent of the total polygenes. In the next screening generation, this percentage is even higher, and with each breeding cycle the levels of horizontal resistance increase until no further increase is either possible or necessary. This process of quantitative increase, in which the progeny have a higher level of a quantitative variable than their parents, is known as transgressive segregation.

So, when breeding for horizontal resistance, there is no need to begin with a good source of resistance, but there must be a reasonably wide genetic base to ensure that all the necessary polygenes are present.

In practice, it is much easier to breed for horizontal resistance than it is to breed for high yield, high quality of crop product or high agronomic suitability. It is therefore best to use high-yielding, high-quality, agronomically suitable, but susceptible, modern cultivars as the original parents. With suitable selection procedures, it should be easy to gradually increase the levels of horizontal resistance, while retaining the other desirable qualities. Conversely, it would be very difficult to use highly resistant, primitive archetypes as the original parents, and then try to improve their various agricultural attributes, while retaining their resistance.

The maize of tropical Africa illustrated this point conclusively. The horizontal resistance accumulated within very susceptible host populations of highly prized local landraces. No 'good source' of resistance was necessary, and no diminution of the prized characteristics occurred. When breeding for horizontal resistance, therefore, the discernible qualities of the genetic resources must be those of yield, quality of crop product and agronomic suitability.

15.4.4 Population breeding

Recurrent mass selection means that a heterogeneous plant population is screened for the best individuals, which then become

the parents of the next generation. This process is repeated some 10–15 times, by which time the upper limits of most quantitative variables will have been reached. In each breeding cycle (i.e. each generation of recurrent mass selection), there should be at least 10 to 20 parents, depending on the nature of the crop. These parents may be randomly cross-pollinated, or hand-pollinated in all combinations, again depending on the nature of the crop.

Quantitative variables change as a result of selection pressures. The term 'pressure' is used in the sense of bringing pressure to bear, of coercion or persuasion, and selection pressures can be positive or negative.

Positive selection pressures lead to the increase of a variable, while negative selection pressures lead to its decrease. The mechanism of these changes is reproductive fitness. For example, if a heterogeneous host population is susceptible to a parasite, the most resistant individuals will be parasitized the least and will reproduce the most, while the most susceptible individuals will be parasitized the most and will reproduce the least. With each generation the population as a whole will gain resistance as a consequence of this positive selection pressure for resistance.

Conversely, if the parasite is absent from the locality in question, as with the maize of tropical Africa, or because of a functioning vertical resistance or the use of a pesticide (i.e. the vertifolia effect; see above), the selection pressure for horizontal resistance will be negative, and the frequency of genes controlling horizontal resistance will decrease. This happens because any unnecessary genetic characteristics tend to decline to a level called the Hardy-Weinberg equilibrium.

Positive selection pressure can be increased by increasing the ratio of selected plants to total plants. This ratio is called the selection coefficient. In practice, this means that the screening population should be as large as possible so that perhaps only one plant in a thousand becomes a parent of the next generation. The possibilities depend very much on the nature of the crop. If the plants are small, such as wheat, rice or beans, it is entirely feasible to use a screening population of some 100 000 plants, but if the population is a tree crop, such as a fruit or nut species, such large populations are not feasible. However, the size of the screening population is not critical, and if a relatively small population is necessary because of land or labour restrictions, the breeding programme will require more time, but the deficiency will be no worse than this.

Should it transpire that the original genetic base was too narrow to accumulate adequate horizontal resistance, new genetic material can be added to the screening population. This may lead to an initial, slight loss of horizontal resistance, but the ultimate potential will be improved.

A special aspect of quantitative variables is that they must all be increased simultaneously. There is little point in having high levels of horizontal resistance to all of the locally important parasites except one. Even a single susceptibility will spoil a cultivar, and make spraying or some other form of artificial control necessary (see also sub-optimization, above). This is a major difference between breeding for single-gene and multiple-gene characters. Pedigree breeding allows the transfer of a single-gene character, such as a resistance, from a wild plant to a cultivar by hybridization and back-crossing. A multiple-gene variable cannot be transferred in this way because hybridization leads to an immediate dilution. Hence the need for

a simultaneous increase of all quantitative variables during population breeding.

Once the required levels of quantitative variables have been reached, steps can be taken to produce pure lines, clones, or synthetic or hybrid varieties, according to the requirements of the crop in question.

The maize of tropical Africa provided good examples of both negative and positive selection pressures to tropical rust. It also illustrated the need for horizontal resistance to all locally important parasites, because subsistence farmers do not use pesticides on their food crops.

15.4.5 Male gametocides

A male gametocide is a chemical that makes a plant male-sterile but female-fertile. By using male gametocides in a screening population, inbreeding plants, such as wheat, can be converted to outbreeders. A wheat population then becomes the equivalent of a maize population, with unsprayed plants acting as male parents. This can be very useful as it eliminates the laborious and severely limiting process of cross-pollinating by hand. Working this way in Brazil, Beek (1988) obtained millions of wheat crosses with only an hour or two of work.

The details of male gametocides are beyond the scope of this chapter and amateur breeders who decide to use these chemicals should get the advice of the specialists who are cooperating in the participatory plant breeding.

15.4.6 Screening existing populations

With some crops, particularly tree species, it is possible to find all the resistance we need by screening existing, heterogeneous populations. This was possible with coffee in Ethiopia (see below). There is then no need for a formal breeding programme as such. Other tree crops in which such an approach is feasible include cocoa (Witch's broom disease caused by *Crinipellis perniciosa*) and rubber (South American leaf blight caused by *Microcyclus ulei*) in the Amazon valley, tea (Blister blight caused by *Exobasidium vexans*) in India, date palms (Bayoud disease caused by *Fusarium oxysporum* f.sp. *albedinis*) in North Africa, coconut (Cadang-Cadang disease) in the Philippines, white pines (Blister rust caused by *Cronartium ribicola*) in North America, and many other plantation forest species.

15.4.7 Negative screening

Negative screening means that you identify the worst individuals in a population and remove them, rather than identifying the best individuals and keeping them. Negative screening can be useful in two situations. The first occurs when there is a danger of cross-pollination from undesirable individuals in a screening population, such as open-pollinated crops, or self-pollinated crops in which the mother plants have been treated with a male gametocide. These undesirable male individuals must be identified and either removed or deflowered.

A similar situation occurs when a heterogeneous population of an open-pollinated annual crop, such as alfalfa, is being improved during the process of seed production. It is often more profitable to remove the relatively few individuals that show the most susceptibility, than it is to collect the individuals that show the most resistance. This negative selection should obviously be conducted before cross-pollination becomes possible.

Negative screening can also be used profitably in a heterogeneous tree crop. For example, a crop of cocoa might be heavily diseased with Witch's broom disease (*Crinipellis perniciosa*). A small percentage of the trees are highly susceptible and

are infecting all the other trees. If the most susceptible trees are identified and removed, and all other diseased branches are also removed, this parasite interference (see above) will stop and the disease will be controlled. Even if the disease is merely reduced in intensity, further negative screenings of the most susceptible trees will eventually control the disease. This procedure is often far more economical than a positive screening for resistant trees, followed by a subsequent replanting of the entire crop with these selections.

15.4.8 On-site selection

The maize of Africa illustrated the importance of on-site selection (see above). *Puccinia polysora* has maximum epidemiological competence at the equator, and at sea level. As latitude increases, the epidemiological competence decreases to nothing at sea level at the tropics of Cancer and Capricorn. As altitude increases, the epidemiological competence decreases to nothing at the equator at elevations of about 1 200 m.

Maize from the highlands of Kenya, where tropical rust lacks epidemiological competence entirely, is extremely susceptible when planted at sea level near the equator. Conversely, maize in Malawi was reported to be highly resistant to tropical rust but it proved to be very susceptible when planted near the equator at sea level. This was environmental erosion that occurred because the maize had come from an area of minimum epidemiological competence of the pathogen, where it had suffered minimum selection pressure for resistance. When planted in an area of maximum epidemiological competence, its susceptibility was revealed.

Because this maize in tropical Africa was cultivated as an open-pollinated crop, each farmer's crop constituted a natural screening population. Each farmer's maize then constituted a landrace with exactly the right amount of horizontal resistance to tropical rust for that latitude and that altitude. This was an example of subconscious selection

Because tropical rust is so sensitive to altitude and latitude, the pathosystems, and hence the agro-ecosystems, of tropical rust are small. However, this extreme of environmental sensitivity is unusual, and with most crop species the agro-ecosystems are quite large, and relatively few breeding programmes are necessary.

15.4.9 A holistic approach

The tropical maize in Africa also illustrates the need for a holistic approach. Before the appearance of tropical rust, the maize had no important pests or diseases. In other words, it had high levels of comprehensive horizontal resistance. That is, it had adequate levels of horizontal resistance to all the locally important parasites. With the introduction of this re-encounter parasite, this pathosystem balance was immediately lost, and it required about a dozen generations of selection to restore it.

It can be argued that pathosystem balance has been lost in virtually all of our modern crops. The objective of participatory plant breeding should be to restore pathosystem balance in each crop species in each agro-ecosystem. When we consider the many different crops and the many different agro-ecosystems worldwide, this is too big a task for professional plant breeders to undertake on their own, and it is perhaps the best justification of participatory plant breeding.

15.4.10 Selection pressures for other qualities

If we were to produce new cultivars that had high levels of horizontal resistance to all

locally important parasites, but which had reduced yield and quality of crop product, we would be sub-optimizing (see above). This is why we should use modern but susceptible cultivars as our genetic resource. It is clear that a good source of resistance is not necessary when breeding for horizontal resistance, but that high yield, quality and agronomic suitability are necessary. The levels of various horizontal resistances are increased while selection pressures for yield and quality are maintained to ensure that these qualities are not reduced.

15.4.11 Measurement of horizontal resistance

When measuring the results of breeding for horizontal resistance, assessments can be relative only. That is, we can say that a new cultivar has either greater or less horizontal resistance to a particular parasite than another well known and well tried cultivar. An alternative description can be given with the phrase 'spraying not necessary', but even this must be qualified with the rider that this is only true in a normal season.

15.4.12 Crops that are difficult or impossible to breed

For technical reasons, some crops are difficult or even impossible to breed, and amateur breeders should not attempt to improve them. These include banana, citrus, date palm, figs, garlic, hops, horseradish, olives, pineapple, sisal and wine grapes. However, most of the main food crops are easy to breed, and none of them could be described as being difficult to breed. Worldwide, it is clearly logical for amateur plant breeders to work with participatory plant breeding of crops that are easy to breed, while the professional plant breeders should work with crops that are difficult to breed.

15.5 EXAMPLES OF BREEDING FOR HORIZONTAL RESISTANCE

Simmonds (1991) has compiled a comprehensive review of the results of breeding for horizontal resistance. He gives examples of durable resistance in 21 species of crop, functioning variously against airborne and soil-borne pathogens—fungal, bacterial, viral, insect and nematode. Stoner (1992) reviewed 705 papers on host resistance to insects and mites in vegetables, and she also quotes reviews of this topic in grain crops, alfalfa and cotton. She comments that, in most studies, the resistance is a quantitative trait, but she adds that there has been little plant breeding for resistance to insects.

15.5.1 Potatoes in Kenya, Mexico, Scotland and the United States of America

John S. Niederhauser was one of the pioneers of horizontal resistance. Indeed, he was the first scientist to reject the use of vertical resistance in favour of horizontal resistance, and he did this in Mexico with resistance to Late blight of potato (*Phytophthora infestans*). His most famous cultivar was Atzimba, and Mexican scientists have continued his work. Blight is so severe in Mexico that the popular cultivar Alpha has to be sprayed with fungicides 25 times each season. The new, horizontally-resistant cultivars, such as Sangema and Tollocan, need to be sprayed only once or twice each season.

Working in Scotland, Simmonds (1976) demonstrated that the potatoes cultivated in Europe (*Solanum tuberosum*) were derived from the *S. andigena* of South America. With only four generations of recurrent mass selection he was able produce '*neo-tuberosum*' from *S. andigena*, and he was also able to accumulate useful levels of horizontal resistance to Late blight.

In Kenya, Robinson (1996) attempted to imitate the maize of tropical Africa when breeding potatoes for horizontal resistance to both Late blight (*Phytophthora infestans*) and Bacterial wilt (*Pseudomonas solanacearum*). He was able to have two breeding cycles each year with 150 000 seedlings in each cycle. When his cultivar Kenya Baraka was released to farmers, the annual potato production of this country increased from less than 10 000 t in 1974, to an estimated 1 million tonnes in 2004. This production was possible without any use of crop protection chemicals, and without any renewal of seed stocks by the use of certified seed tubers. It should be noted, however, that the temperate viruses of potato lack epidemiological competence in this country, and the Colorado beetle (*Leptinotarsa decemlineata*) is absent.

In the United States of America, Fisher, Deahl and Rainforth (2002) have been breeding potatoes for horizontal resistance to Colorado beetle (*Leptinotarsa decemlineata*) of potatoes and have made useful progress after only a few generations of recurrent mass selection. No single-gene resistances occur against this insect parasite, and this is apparently the first serious attempt to breed for resistance to it in more than a century.

15.5.2 Coffee in Ethiopia

Arabica coffee (*Coffea arabica*) is apparently an allo-tetraploid that was derived from two diploid species in the area of modern Uganda in about 650 CE. It soon died out in its centre of origin, but it was taken at an early date to Ethiopia, which became the centre of diversification. A pathogen (*Colletotrichum coffeanum*) of modern coffee, which causes Coffee berry disease, was left behind, and Ethiopia remained free of this disease until 1970, when this re-encounter parasite was inadvertently introduced. The coffee crops of Ethiopia were heterogeneous, and trees with the minimum horizontal resistance lost all their berries three months before harvest. Trees with the maximum horizontal resistance lost no berries at the time of harvest, and they occurred with a frequency of about one in a thousand. The overall effect was an average yield loss of 40 percent, and this destroyed the economic viability of this crop.

About half-a-million trees were examined and 650 resistant individuals were identified. Their first harvests were kept for seed, and about 1 000 seedlings were germinated from each tree. The first screening criterion was for homozygosity, and only those trees that were 'breeding true' were kept. (*Coffea arabica* is self-pollinating, with about 3 percent of out-crossing). Other tests included yield, cup quality and resistance to other pests and diseases. The best 25 lines became available as new cultivars for farmers only eight years after the programme was initiated (Robinson, 1996).

15.5.3 Beans in Mexico

Roberto Garcia Espinosa has been using recurrent mass selection on black or common beans (*Phaseolus vulgaris*) in Mexico with a view to increasing the levels of horizontal resistance to all locally important parasites. Commercial crops will yield up to 1 500 kg/ha if they are properly protected with fungicides and insecticides. Beans from the seventh breeding cycle of the recurrent mass selection programme yield 2 400 kg/ha without any use of crop protection chemicals. This work has yet to be scientifically described (Roberto Garcia Espinosa, pers. comm., 2007).

15.5.4 Sugar cane in Hawaii

For many years, the sugar cane breeders of Hawaii differed from all other cane

breeders in that they used recurrent mass selection. They produced about three million seedlings in each breeding cycle. These would be reduced to about 600 000 on the basis of visual appearances only. The survivors would be tested for sucrose content, with further massive reductions in numbers. As the number of survivors decreased, the complexity of the tests could be increased. The final result is that Hawaiian sugar cane yields twice as much as any other country, and it does this without any use of crop protection chemicals, other than to protect the cut surfaces of the cane setts used for planting.

15.5.5 Sweet potatoes in United States of America

Jones, Dukes and Cuthbert (1976) were among the pioneers of horizontal resistance breeding when they worked with sweet potato (*Ipomoea batatas*) in South Carolina. They used recurrent mass selection and accumulated good levels of horizontal resistance to several species of insect pests and fungal parasites, as well as improvements in yield and quality. This is an easy crop to work with as it possesses no vertical resistance genes.

15.5.6 Wheat in Brazil

Beek (1988) attempted recurrent mass selection with wheat in Brazil, using male gametocides to achieve large numbers of random cross-pollinations. He used hydroponics and single-seed descent to allow late selection. He made good progress in accumulating horizontal resistance to a number of wheat parasites but he was unable to complete his programme. Specialists advising amateur breeders in a participatory wheat breeding project should regard Beek's report as essential reading.

15.6 ANCIENT CLONES

Ancient clones obviously have high levels of horizontal resistance to all their parasites, and this resistance has endured for centuries, even millennia, in crops such as aroids, bananas, dates, figs, garlic, ginger, hops, horseradish, olives, peppercorns, pineapple, saffron, sisal, turmeric, vanilla, wine grapes and yams. Some of these clones, such as wine grapes, dates and bananas, are now severely parasitized in some areas, but this is only because of new-encounter, foreign parasites.

15.7 AUTOCRATIC AND DEMOCRATIC PLANT BREEDING

Breeding for vertical resistance is highly technical, expensive, difficult, and repetitious. It usually requires a team of scientists working in a large institute. Inevitably, given these problems, fewer cultivars are produced than with population breeding, and it is important that these cultivars have a wide agro-ecological adaptability so that they can be used over as wide an area as possible. Vertical resistance usually has a very wide adaptability and, coupled with its complete control of a parasite, this makes it an attractive plant breeding approach.

Vertical resistance breeding was typical of the green revolution, and the high yielding, 'miracle' wheats and rices. While there is no question that the increased yields of these cultivars have saved about a billion human lives, the fact remains that farmers had little choice of cultivar because there were relatively few of these high-yielding cultivars available. A further disadvantage was that those cultivars that were available were liable to fail when their vertical resistances broke down. Breeding for vertical resistance also makes farmers totally dependent on the formal seed system.

This approach might be termed 'autocratic' plant breeding, because it is the

breeder, rather than the farmer, who decides what kind of cultivar is to be bred, and which cultivar is to be grown. Its converse is 'democratic' plant breeding, in which the farmer participates in the breeding, has a choice of resistance type, has a wide choice of cultivars, and can make their own decisions concerning which cultivars to grow. Democratic plant breeding is possible with horizontal resistance, which is so easy to use that it can be employed by numerous amateur breeders, who cooperate with professionals in participatory plant breeding and plant breeding clubs.

15.7.1 Plant breeding clubs

Plant breeding clubs are made up of amateur breeders, who might be hobby gardeners, environmentalists, green activists, farmers, students or even schoolchildren. Each club is independent and free to breed any crop it chooses, for any improvements it chooses and using any breeding methods it chooses. Their primary objective is likely to be the production of new cultivars with sufficient horizontal resistance to permit cultivation without any crop protection chemicals, and without any reduction in yield, quality or agronomic suitability. This is because breeding for durable, horizontal resistance is so easy when compared with breeding for the ephemeral, but complete, vertical resistance.

Plant breeding clubs are particularly useful with participatory plant breeding. A group of sympathetic crop specialists can cooperate with several plant breeding clubs, consisting of various categories of amateur breeders. Possibly the most effective clubs are university plant breeding clubs made up of student-breeders assisted by professors. Greater details have been provided by Robinson (2004).

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CHAPTER 16

Breeding for quantitative variables Part 3: Breeding for resistance to abiotic stresses

Stefania Grando and Salvatore Ceccarelli

16.1 INTRODUCTION

Plant breeding has been very successful in environments that are either naturally favourable or that can be made profitably favourable by irrigation and fertilizer and by chemical control of pests and diseases.

Cox *et al.* (1988) estimated that the annual genetic gains in bread wheat in the United States of America from 1917 to 1987 have been 16 kg/ha/yr. Russell (1984) found that the genetic gain in maize hybrids released between 1930 and 1980 was 54.2 percent. Austin, Ford and Morgan (1989) estimated 38 kg/ha/yr for the genetic gain between 1908 and 1985 in wheat in the United Kingdom. Similar examples are available in many other crops.

By contrast, yield improvements have been very elusive in marginal environments, to the extent that the role of breeding for those environments is often questioned. What it is not questioned is why it has not been possible to improve agricultural production by simply transferring into marginal environments cultivars or methodologies that have made breeding for favourable conditions so successful. As a result, the yield of some important staple crops has shown only modest increases or remained virtually unchanged (Ceccarelli and Grando, 1996; Ceccarelli *et al.*, 2004). This has been attributed to the difficult nature of the target environments where yields have shown little increase (Passioura, 1986; Blum, 1988) and has been accepted as inevitable. Therefore, most of the selection work in breeding programmes is done in favourable conditions (Simmonds, 1991), and much research has been done, and resources expended, to seek alternatives to empirical breeding for unfavourable conditions, such as analytical breeding and, more recently, molecular breeding. Much less has been done on assessing whether a paradigm shift was needed when selecting for abiotic stresses.

One hypothesis is that cultivars often defined as 'widely adapted' are actually specifically adapted to conditions that are at or near the optimum for crop growth. Therefore the superiority they have in these environments is lost in suboptimal environments.

The objective of this chapter is to discuss critical problems associated with breeding for abiotic stresses, to analyse possible reasons for the limited success breeding has had in stressed environments, and to indicate that participatory plant breeding is one way to overcome the inherent difficulties. Most examples are derived from ICARDA's barley breeding programme for low-rainfall areas.

16.2 MOST COMMON ABIOTIC STRESSES

Abiotic stresses are consequences of extremes of physical environment comprising climatic stresses, such as drought, flood, heat and cold; and soil or water conditions, such as salinity, metal toxicity and nutrient deficiency. Plants can experience abiotic stresses resulting from the shortage of an essential resource, or from a toxic excess of a substance, or from climatic extremes. In some cases the same resource can impose stress both when in shortage and when in excess (i.e. water and temperature). Occurrence, severity, timing and duration of stresses vary from location to location, and in the same location from year to year. In the case of drought, cultivars successful in one dry year may fail in another, or cultivars resistant to terminal drought may not be resistant to intermittent drought, or to drought occurring early in the season (Turner, 2002). In addition, abiotic stresses seldom occur in

isolation; they often interact, both with other abiotic stresses and with biotic stress. Moreover, areas with a high probability of abiotic stresses generally have low-input agriculture (Cooper *et al.*, 1987), because the risk of losing the crop or of a low yield discourages the farmers from using costly inputs, particularly fertilizers. This results in low outputs, poor human nutrition and reduced educational and employment opportunities, especially for girls. The rural poor are particularly badly affected because of lack of access to alternative sources of employment or food.

16.2.1 Drought

Drought, defined as water availability below that required for maximum crop yield, is one of the main factors limiting crop production. Although it reaches the front pages of the media as drought warnings or when it causes famine and death, drought is a permanent constraint to agricultural production in many developing countries, and an occasional cause of losses in agricultural production in developed ones. Several drought warnings have been issued in recent years in Australia, Europe and the United States of America. Climate changes will increase the frequency of droughts, particularly in Southeast Asia and Central America, and by 2050 are expected to cause water shortages for 67 percent of the future population in the world (Ceccarelli *et al.*, 2004).

In areas where water availability is limited, and irrigation is not available, the choice of crops is restricted to a few, and often to only one, thus making farmers in those areas vulnerable for lack of options. In fact, most of the rural poor live in areas where crop productivity and crop diversification are limited by lack of water. Therefore it is not surprising that there is an ongoing global research effort on social, agronomic, genetic, breeding, physiological and molecular aspects of drought resistance, or as recently more often used, water productivity (Passioura, 2006). This is highlighted by the publication of several reviews (Ceccarelli *et al.*, 2004; Reynolds, Mujeeb-Kazi and Sawkins, 2005; Parry, Flexas and Medrano, 2005).

Drought has been always a challenge to plant breeders, despite many decades of research (Blum, 1993). The development, through breeding, of cultivars with higher and stable harvestable yield under drought conditions would be a major breakthrough (Ceccarelli and Grando, 1996). However, drought resistance is a very elusive trait from a genetic point of view. This is because the occurrence, severity, timing and duration of drought vary from year to year, and although every year there are "winners", it is difficult to find those that are consistently successful. To make matters worse, drought seldom occurs in isolation; it often interacts with other abiotic stresses (particularly temperature extremes), and with biotic stress. As mentioned earlier in this chapter, the risk of losing the crop because of drought limits the use of inputs.

Also the definition of dry areas seems to be an elusive issue. This is illustrated by the distribution of crops in different agroclimatic environments. For example, in a country such as the Syrian Arab Republic, with a large spatial variability of rainfall within short distances (van Oosterom, Ceccarelli and Peacock, 1993), bread wheat (*Triticum aestivum* L.), durum wheat (*T. turgidum* var. *durum* L.) and barley among the cereals, and faba bean (*Vicia faba* L.), chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* L.) among the food legumes, are grown in progressively drier environments, with some overlapping. Therefore, a dry area for faba bean or bread

wheat is moderately favourable for durum wheat and chickpea, and a dry area for durum wheat and chickpea is moderately favourable for barley and lentil.

At the drier end of the spectrum, barley and lentil are the only rainfed crops, and the other cereals or legumes are only grown under supplementary or full irrigation. The situation described for the Syrian Arab Republic applies to most countries of the Mediterranean basin and West Asia, and for crops such as millet, sorghum and maize to the dry areas of the tropics, and is only altered by irrigation.

The complexity of breeding for dry areas is not only due to the biological complexity of drought resistance, but also to the consequences of drought for the livelihood of people living in the dry areas. In developed countries, farmers have various forms of social protection against the devastating effects of drought, while in developing countries farmers have to survive on their own, usually selling their assets, most commonly livestock. In areas affected by drought in developed countries farmers may prefer cultivars capable of high yields in the few favourable years; this is very different in the dry areas of most developing countries with no or little social assistance where farmers prefer varieties capable of some yields even in the driest years. This is an example that the same biological problem in different social contexts requires different solutions.

16.2.2 Soil toxicities and deficiencies

Soil plays a major role in determining the amount and availability of nutrients and toxic minerals. Soil toxicities and deficiencies render more than one hundred million hectares of agricultural land marginal for agriculture, limiting production and creating poverty for millions.

Soil mineral stresses are increasingly becoming important limiting factors for crop plants in many parts of the world. Acid soil and associated aluminum toxicity affect over 2 billion hectares worldwide (Humphreys and Humphreys, 2005). Mineral nutrient deficiency can be caused by low nutrient status of the soil, low mobility or availability of nutrients within the soil.

Salinity is generally defined as the presence of excessive amount of soluble salts that hinder or affect the normal function of plant growth (Shafiq-ur-Rehman, Harris and Ashraf, 2005). Saline soils have a mixture of chloride salts, with sodium chloride being often dominant. Salinity can be divided into primary sources in soils derived from saline parent rocks (Sposito, 1989) and secondary salinization caused by human intervention, such as irrigation (Sposito, 1989).

Salinization is one of the most common forms of soil degradation. Almost all continents have problems related to saline soils (Pessarakli, 1999), and is particularly severe in arid and semi-arid regions.

It is estimated that 6 percent of the world's land and 30 percent of the world's irrigated areas already suffer from salinity problems (Unesco Water Portal, 2007).

16.2.3 Temperature stresses

Temperature extremes can be experienced on both a daily or seasonal basis. Long-term climatic changes lead to higher average temperatures and increase the frequency and severity of extreme temperature events. As with other stresses, early and late stages of crop growth are particularly sensitive to temperature extremes. Plants can be affected by exposure to prolonged periods of moderately high temperatures as well to short periods of extremely high tempera-

tures. Low temperatures can affect plants by chilling, which leads to physiological and developmental abnormalities, and by freezing, which causes cell damage. About 15 percent of arable land is estimated to be affected by freezing stress (Dudal, 1976).

Changes in temperature are the most certain aspect of climate changes. The most recent evidence from the Fourth Assessment Report on Climate Change of the Intergovernmental Panel on Climate Change (IPCC), published in 2007, indicates that the warming of the climate system is unequivocal, as it is now evident from observations of increases in global average air and ocean temperatures, widespread melting of snow and ice, and rising global average sea level. This is shown by (i) 11 of the last 12 years (1995–2006) rank among the twelve warmest years in the instrumental record of global surface temperature (since 1850); (ii) the temperature increase is widespread over the globe, and is greater at higher northern latitudes; (iii) global average sea level has risen since 1961 at an average rate of 1.8 mm/yr, and since 1993 at 3.1 mm/yr, with contributions from thermal expansion, and melting glaciers, ice caps and the polar ice sheets; and (iv) observed decreases in snow and ice extent are also consistent with warming. Satellite data since 1978 show that annual average Arctic sea ice extent has shrunk by 2.7 percent per decade, with larger decreases in summer of 7.4 percent per decade. Mountain glaciers and snow cover on average have declined in both hemispheres.

The 2007 report indicates that it is also very likely that over the past 50 years, cold days, cold nights and frosts have become less frequent over most land areas, and hot days and hot nights have become more frequent, and it is likely that heat waves have become more frequent over most land areas, the frequency of heavy precipitation events has increased over most areas, and since 1975 the incidence of extreme high sea level has increased worldwide.

In conclusion, higher temperatures are part of the future climate for which breeders should breed today.

16.3 CHALLENGING CONVENTIONAL BREEDING CONCEPTS

Most plant breeders assume that it is too slow and too difficult to breed for environments where droughts or other stresses are unpredictable and variable. The target is hard to define, and heritability, and hence response to selection, is too low to achieve meaningful results. Therefore most of the breeding for stress environments has been actually conducted using the same basic approach that has been very successful in areas where lack of water or other abiotic stresses is seldom important.

With few exceptions, most breeding programmes share the following concepts:

- selection has to be conducted under the well-managed conditions of research stations. It is felt that environmental noises can be kept under control, error variances are smaller and response to selection higher;
- cultivars must be genetically homogenous (pure lines, hybrids, clones) and must be widely-adapted over large geographical areas;
- locally-adapted landraces must be replaced because they are low yielding and disease susceptible;
- seed of improved cultivars must be disseminated through mechanisms and institutions such as variety release committees, seed certification schemes and governmental seed production organizations; and

- the end users of new varieties are not involved in selection and testing; they are only involved at the end of the consolidated routine (breeding, researcher-managed trials, verification trials), to verify if the choices made for them by others are appropriate or not.

Breeders have very seldom questioned these assumptions. When they have, it has been found that:

- selection in well-managed research stations tends to produce cultivars that are superior to local landraces only under improved management—not under the low-input conditions typical of the farming systems of stress environments. The result is that although many new varieties outyield local landraces on a research station and some are released, few if any are actually grown by farmers in difficult environments;
- poor farmers in stress environments tend to maintain genetic diversity in the form of different crops, different cultivars within the same crop or heterogeneous cultivars, or combinations, to maximize adaptation over time (stability), rather than adaptation over space (Martin and Adams, 1987a). Diversity and heterogeneity serve to disperse or buffer the risk of total crop failure due to environmental variation. This is in sharp contrast to the trend of modern breeding towards uniformity;
- resource-poor farmers seldom use the formal seed-supply systems. They frequently rely on their own or on neighbours' seed (Almekinders, Louwaars and de Bruijn, 1994). Therefore, when the appropriate cultivar is selected, adoption is much faster through non-market methods of seed distribution (Grisley, 1993); and
- when farmers are involved in the selection process, their selection criteria may be very different from those of the breeder (Hardon and de Boef, 1993; Sperling, Loevinsohn and Ntabomvura, 1993). Typical examples are crops used as animal feed, such as barley, where breeders often use grain yield as the sole selection criterion, while farmers are usually equally concerned with forage yield and the palatability of both grain and straw.

Although the chapter is largely based on the strategies and methodologies developed during the last 20 years in the ICARDA barley breeding programme, we believe that the main findings have general applicability. They will be described to demonstrate that it is indeed possible to improve the production of a typically low-input crop such as barley, grown in environments with low and poorly-distributed rainfall, low temperatures in winter, high temperatures and drought during grain filling, low soil fertility and poor agronomic management. The data were mainly obtained from three locations in the northern Syrian Arab Republic (Tel Hadya, Breda and Bouider). They represent three distinct agricultural systems. Tel Hadya (348 mm average annual rainfall) is a favourable high-input environment that lends itself to a wide choice of different crops. Bouider (236 mm average annual rainfall) represents the opposite extreme: a typical low-input, high-risk environment where barley is the only possible rainfed crop. Breda (273 mm average annual rainfall) is intermediate between the two, located on the edge of the area where Arabi Aswad becomes the dominant landrace. The three sites are geographically close, located 35 (Tel Hadya), 60 (Breda) and 80 km (Bouider) south-east of Aleppo. The key aspects of these strategies and methodologies are: (i) direct selection for specific adaptation in the target

environment (Chapter 9 in this volume); (ii) use of locally-adapted germplasm; (iii) use of plot techniques and experimental design to control environmental variation (Chapter 3); (iv) participation of farmers in selection; and (v) reliance on the informal seed-supply system to make the seed of new cultivars available to farmers (Chapter 21).

16.4 TYPE OF GERMPLASM

In breeding for resistance to abiotic stresses there are certain types of germplasm—landraces, wild relatives or wild progenitors—that, although of limited or no value in breeding for favourable, potentially high-yielding conditions, may play a fundamental role in the success of a breeding programme. In many developing countries, landraces (also called farmers' varieties, old cultivars or primitive cultivars) are still the backbone of agricultural systems in unfavourable environments (Ceccarelli, 1984; Grando, von Bothmer and Ceccarelli, 2001). In these environments, the replacement of these cultivars has proved to be a difficult task. The reasons why farmers still prefer to grow only landraces or continue to grow landraces even after partial adoption of modern cultivars are not well documented, but include quality attributes such as food and feed quality, and seed storability (Brush, 1999). Landraces are often able to produce some yield even in difficult conditions, whereas modern varieties are less reliable. For example, where farmers have adopted modern cultivars they also have retained the landraces on the most unfavourable areas of the farm (Cleveland, Soleri and Smith, 2000).

Landraces of self-pollinated species are mixtures of a great number of homozygote genotypes (Brown, 1978, 1979; Ceccarelli and Grando, 1999; Grando, von Bothmer and Ceccarelli, 2001). Such evidence is available in many crops, such as lentil (Erskine and Choudhary, 1986), sorghum (Blum, Golan and Mayer, 1991), bread and durum wheat (Porceddu and Scarascia Mugnozza, 1984; Damania and Porceddu, 1983; Spagnoletti-Zeuli, De Pace and Porceddu, 1984; Damania, Jackson and Porceddu, 1985; Lagudah, Flood and Halloran, 1987; Blum *et al*., 1989; Elings and Nachit, 1991), beans (Martin and Adams, 1987a, 1987b), barley (Ceccarelli, Grando and van Leur, 1987; Weltzien, 1988; Asfaw, 1989; Weltzien and Fishbeck, 1990) and others. Therefore landraces contain a large amount of readily-usable genetic variation. Selection within landraces is one of the easiest, oldest and cheapest methods of plant breeding. In most cases, any interest shown by researchers in the variability of landraces has been academic; we know of few cases in which the variability has actually been used in breeding programmes. Yet, as mentioned before, the use of the variability of landraces in the area where landraces are adapted is a cheap and easy way to make progress.

At ICARDA we have used a large collection of barley landraces made in 1981 in the Syrian Arab Republic and Jordan (Weltzien, 1982). The collection was made by visiting the fields of 70 farmers and collecting 100 individual heads in each field. Cvs. Arta, Tadmor and Zanbaka are three examples of pure lines identified so far from two widely grown Syrian barley landraces (Tables 16.1 and 16.2).

In developed countries, landraces have been the basic material for genetic improvement in many crops until about 50 years ago. In these countries, however, the identification of superior genotypes has probably led many breeders to concentrate their attention on those few genotypes and this has resulted in: (i) the use of relatively

TABLE 16.1
Grain yield (t/ha) of Tadmor and Zanbaka in 11 and 8 locations respectively in the northern Syrian Arab Republic

Year	Location (Province)	Arabi Aswad	Tadmor	Zanbaka
1991	Shurkrak (Raqqa)	0.220	0.130	-
	Al Ayouj (Raqqa)	0.260	0.270	-
	Beer Asi (Raqqa)	0.180	0.170	-
1992	Bylounan (Raqqa)	0.640	0.940	1.100
	Masadeih (Hassake)	1.350	1.600	1.330
1993	Bylounan (Raqqa)	0.792	1.176	1.132
	Shurkrak (Raqqa)	0.666	1.268	0.916
1994	Bylounan (Raqqa)	0.360	0.575	0.530
	Al Wastah (Raqqa)	0.560	0.570	0.650
	Tell Hamzeh (Hassake)	0.812	0.876	1.250
	Al Hamar (Hassake)	1.100	1.000	0.650
Mean		0.631 (0.780)	0.780	0.945
% increase over Arabi Aswad		23.6	22.4	

Notes: The mean in parentheses is calculated from the locations in common with Zanbaka. The data are from trials conducted in farmers' fields, without fertilizer.

TABLE 16.2
Grain yield (t/ha) of Arta in 51 locations over seven cropping seasons

Year	No. of sites	cv. Arta	cv. Arabi Abiad	% increase
1986–1987	1	3.738	2.929	27.6
1988–1989	5	1.814	1.530	18.6
1989–1990	8	2.044	1.747	17.0
1990–1991	11	2.388	2.187	9.2
1991–1992	17	3.336	2.455	35.9
1992–1993	5	2.551	1.958	30.3
1993–1994	4	1.160	1.069	9.4

Note: The data are from trials on farmers' fields in the Syrian Arab Republic (except those of 1986–1987, which are from Breda research station).

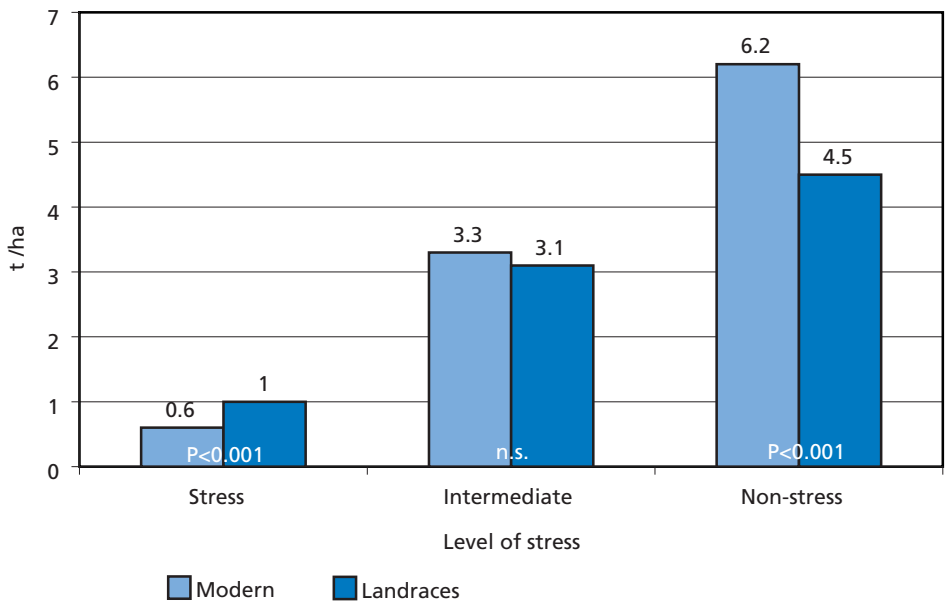
few parents, leading to a considerable reduction of genetic diversity; and (ii) the loss of most of the landraces before they could be collected and conserved in germplasm banks, and (probably) before assessing whether their potential had been fully exploited.

The value of landraces as sources of drought tolerance is well documented in the case of barley in the Syrian Arab Republic (Grando, von Bothmer and Ceccarelli, 2001; Comadran *et al.*, 2008; Pswarayi *et al.*, 2008) and in several other crops elsewhere (Brush, 1999).

The comparison between barley landraces and modern cultivars under a range of conditions, from severe stress (low input and low rainfall) to moderately favourable conditions (high inputs and high rainfall), has consistently indicated that:

- landraces yield more than modern cultivars under low-input and stress conditions (Figure 16.1);
- the superiority of landraces is not associated only with mechanisms to escape drought stress, as shown by their heading date;

FIGURE 16.1
Grain yields (t/ha) of pure lines derived from Syrian landraces and modern cultivars at three levels of stress in the Northern Syrian Arab Republic



Source: Ceccarelli, 1996.

- within landraces there is considerable variation for grain yield under low-input and stress conditions, but all the landrace-derived lines yield something, while most modern cultivars fail;
- landraces are responsive to both inputs and rainfall and the yield potential of some lines is high, though not as high as modern cultivars; and
- it is possible to find modern cultivars that under low-input and stress conditions yield almost as well as landraces, but their frequency is very low.

The data in Figure 16.1 also suggest that selection conducted only in high-input conditions is likely to miss most of the lines that would have performed well under low-input conditions. Figure 16.1 also shows that the assumption of most breeding programmes that landraces are genetically inferior is based on work conducted in research stations. Even those breeding programmes addressing target environments that have low yield potential because of the combination of biotic and abiotic stresses have rarely challenged this assumption.

The superior performance of landraces in dry areas is also evident from the frequency with which they are selected by farmers (Figure 16.2). The data in Figure 16.2 also show the importance and the role that the wild relatives, in this case the wild progenitor of cultivated barley, *Hordeum spontaneum*, have as a source of resistance to extreme levels of drought (Grando, von Bothmer and Ceccarelli, 2001; Ceccarelli *et al.*, 2004).

Gas exchange observations made at anthesis in a wet site showed that *H. spontaneum* had widely open stomata,

FIGURE 16.2
Frequency of selection by farmers of four types of germplasm (modern, landraces, landraces × modern and landraces × *Hordeum spontaneum*)

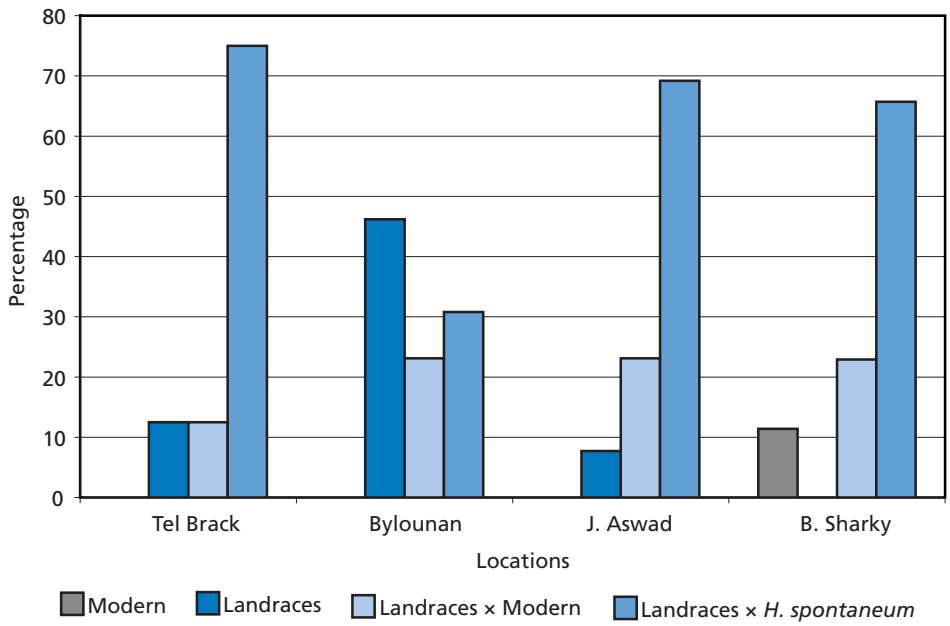


TABLE 16.3
Gas exchange parameters of *Hordeum spontaneum* accessions (means of 12 accessions) and ratio *H. spontaneum/H. vulgare* at Tel Hadya, Syrian Arab Republic

Parameter	Units	*H. spontaneum*	*H. spontaneum/H. vulgare*
Net photosynthesis	$\mu mol\ m^{-2}\ s^{-1}$	16.90 ± 0.80	1.49
Leaf conductance	$mol\ m^{-2}\ s^{-1}$	0.40 ± 0.03	3.57
Transpiration efficiency		4.35 ± 0.09	0.63
Leaf temperature	C°	25.50 ± 0.36	0.85
Pre-dawn leaf water potential	Mpa	0.89 ± 0.25	1.35

higher net photosynthesis and lower pre-dawn leaf water potential at this stage of development than did cultivated barley (Table 16.3). The ability of some accessions of *H. spontaneum* to tolerate extreme levels of drought stress was evident during the severe drought of 1987, when two lines of *H. spontaneum* were the only survivors in the breeding nurseries grown at Bouider (Syrian Arab Republic), which had received only 176 mm rainfall (Grando, von Bothmer and Ceccarelli, 2001).

These lines had some photosynthetic activity early in the morning, even though six times less than in absence of stress, stomata were open and the pre-dawn leaf water potential was negative. At the same time, the stomata of the black-seeded local landrace, Arabi Aswad, considered by farmers to be very resistant to drought,

were closed, even though the pre-dawn leaf water potential was slightly higher than in *H. spontaneum*. By midday, the stomatal conductance of *H. spontaneum* decreased and net photosynthesis became negative, while the stomatal conductance of Arabi Aswad was zero.

16.5 BREEDING METHODS

Individual plant selection (such as in the widely used 'pedigree method') in crops that are normally grown in dense stands is very effective for traits that are not affected by competition. The best example is probably disease resistance. However, many characters of interest to the breeder are strongly affected by competition. Among others, the ability to tolerate water stress is certainly greatly affected by the distance between plants competing for limited available water. The result is that isolated plants (such as those of a spaced-plant F_2 used in the pedigree method) grow much better than they would if planted at normal density. The argument that this does not matter as long as all plants are in the same conditions ignores the possible effects of genotype × competition interaction.

One breeding method that, in the case of self-pollinated crops, seems particularly suitable to breeding for resistance to abiotic stresses is the bulk-pedigree method, in which, after producing the F_1 and the F_2 on station, three years of multilocation yield testing and selection of the bulks are carried out in the target environment(s). Selection is done between bulks by identifying the best populations for either yield or other characters. In parallel with the field testing of the bulks, a within-bulks selection is conducted only in those bulks that are selected for the next level of field testing: 10 to 50 heads are collected from the selected populations. The progenies of the selected heads are grown as head rows and tested for disease resistance or quality characteristics. Some bulks will lose the superiority shown the year before because of genotype × environment interaction and because of decreasing heterozygosity and associated reduced heterotic effects. The corresponding families will also be discarded.

The families deriving from the populations that maintained their superiority for three cropping seasons will enter yield testing.

When the programme is fully implemented, the yield trials contain two types of materials: new bulks, and pure lines derived from the superior bulks of the previous cycle. If the requirements for the genetic uniformity of the varieties to be released in a given country are very strict, only the pure lines will considered as candidates for release.

The method is based on the basic assumptions that (i) a superior bulk is made by a large number of superior genotypes, and (ii) that if the superiority is maintained for a period of three cropping seasons in a highly variable environment, the probability is small that the superiority is associated with heterosis. The method can also be used to test the importance of population buffering in relation to stability.

The method is based on the exploitation of the genetic variance between populations (V_b) because estimates of V_b are comparatively easy and economical to obtain, while estimates of within-population variance (V_w) are more expensive and much less precise because of interaction and competitive effects (Simmonds, pers. comm.).

This method has proved to be ideal for use in participatory breeding programmes with self-pollinated crops (Ceccarelli and Grando, 2007).

16.6 SELECTION CRITERIA

The interest in selecting for traits other than yield in plant breeding programmes aimed at increasing crop production is motivated by the difficulties inherent in selecting directly for yield. Literature on the inheritance of yield in several crops has led to the conclusion that yield is inherited in a complex manner (Blum, 1988). In spite of the widespread reference to 'yield genes', it is evident that yield as such is not under 'direct genetic control'. Rather, it is the multitude of physiological and biochemical processes—the integrated effect of which is measured as yield—that are under genetic control (Blum, 1988). The complex manner of inheritance of yield is evident from the generally low estimates of heritability that are of common occurrence for characters that are under complex genetic control. The difficulties of selecting for yield become even greater in environments characterized by unpredictable variability in the frequency, timing and severity of a number of climatic stresses.

Breeding for drought resistance based on putative traits (defined as traits associated with drought resistance, but easier to select for than grain yield) has been, and still is, very popular (Richards *et al.*, 2002).

The ideal trait to be used as an additional or alternative selection criterion to yield in breeding for stress conditions should satisfy the following requirements: (i) be causally related or genetically linked to yield under stress conditions; (ii) exhibit genetic variation; (iii) be highly heritable; and (iv) be easy, inexpensive and quick to screen for.

Traits that have been investigated include physiological and biochemical traits (such as osmotic adjustment, proline content, stomatal conductance, epidermal conductance, cell membrane stability, cell wall rheology, canopy temperature, relative water content, leaf turgor, abscisic acid content, transpiration efficiency, water-use efficiency, carbon isotope discrimination and re-translocation), and developmental and morphological traits (such as leaf emergence, early growth vigour, leaf area index, leaf waxiness, stomatal density, tiller development, flowering time, maturity rate, vernalization requirement and root characteristics).

In the case of barley, traits more consistently associated with higher grain yield under drought are growth habit, early growth vigour, earliness, plant height under drought, long peduncle and a short grain-filling duration (Acevedo and Ceccarelli, 1989). Three traits that deserve a special mention are leaf epidermal conductance (Sinclair, 2000), osmotic adjustment (Serraj and Sinclair, 2002) and desiccation tolerance (Ramanjulu and Bartels, 2002), which appear related to survival under severe stress conditions. Even though the low yields resulting from survival traits may look irrelevant from the perspective of high-input agriculture, they are crucial to the livelihood of farmers in some of the driest regions of the world.

While the analytical approach has been very useful in understanding which traits are associated with drought tolerance and why, it has been less useful in actually developing new cultivars showing improved drought resistance under field conditions. Under such conditions, drought varies in timing, intensity and duration, and therefore it is the interaction among traits to determine the overall crop response to the variable nature of the drought stress rather than the expression of any specific trait (Ceccarelli, Acevedo and Grando, 1991). A typical example is offered by early growth vigour, a trait that is unanimously considered important in reducing the amount of water lost by evaporation from the soil

surface, and therefore in increasing water-use efficiency (Richards *et al*., 2002) in crops grown on current rainfall (absence of stored moisture). The study of barley landraces from the Syrian Arab Republic has revealed that genotypes with a modest early vigour can successfully achieve the same result with a prostrate growth habit (Ceccarelli and Grando, 1999).

Furthermore, many of the studies on putative traits have been conducted independently from, or as a side-activity to, breeding programmes, and by non-breeders. As a consequence, in general, breeders have taken a sceptical attitude towards these studies, with the well-founded justification that the stated conclusions are affected by either the low number of genotypes involved, or the particular type of germplasm used or the insufficient number of environments. This attitude emerges clearly even in the case of individual-trait breeding to enhance genetic yield potential (Rasmusson, 1987).

Breeding for drought resistance based on direct selection for grain yield in the target environment (empirical or pragmatic breeding) appears intuitively to be the most obvious solution. However, it has faced the major criticism that since field-drought is such a moving target, the chances of progress appear slow at best and possibly remote. One major consequence of this attitude has been to study a less mobile target by simulating drought in laboratory (or greenhouse) conditions, which results in generally irrelevant shocks (Passioura, 2002). Several studies have been and are being conducted addressing 'laboratory drought' with the main justification being to discover mechanisms and genes (Yamaguchi-Shinozaki and Shinozaki 1994; Kasuga *et al*., 1999; Nakashima *et al*., 2000; Seki *et al*., 2001).

While there is currently substantial investment in molecular approaches to the study of drought resistance, there are not yet success stories based on the identification of specific genes and their utilization for this challenge (Chapman, 2008).

Ultimately it is the drought resistance under field conditions that needs to be improved. Yield under stress conditions continues to be the major selection criterion. In the case of barley, additional selection criteria utilized are early growth vigour, plant height under stress, tillering and earliness.

16.7 ARCHITECTURE OF GENOTYPES AND YIELD STABILITY

One of the most dramatic changes introduced by modern agriculture has been reduction of variability. The narrowing of the genetic base that has been a feature of plant breeding in developed countries has been accompanied by a trend towards homogeneity: one clone, one pure line, one hybrid (Simmonds, 1983). Uniformity and broad adaptation are very useful attributes to accommodate large-scale centralized seed production (Davis, 1990). While this trend is now being questioned in developed countries (Wolfe, 1992), it is still very common in breeding programmes for developing countries at both national and international levels.

In breeding programmes aiming at increased stability, the problem of reduced variability is particularly serious in relation to the two major genetic mechanisms promoting stability: individual buffering and population buffering. Individual buffering is largely a property of heterozygotes, and although there is some evidence of individual buffering not associated with heterozygosity, it may be difficult to exploit this mechanism in self-pollinated

diploid crops (Allard and Bradshaw, 1964). However, as modern varieties of cereal crops such as wheat and barley are mostly pure lines, they must rely on individual buffering to be stable. Population buffering is a mechanism of stability associated with genetic heterogeneity. 'Varieties' made up of a number of genotypes, such as the landraces, are well buffered (stable), because each member of the population is best adapted to slightly different conditions from other members of the population. The stability of the individuals is sacrificed to maximize the stability of the population. Although a direct relationship between genetic heterogeneity and stability has yet to be demonstrated for landraces, it can be speculated that, being the product of natural and artificial selection following domestication, the genetic structure of landraces must bear some advantage, or at least cannot be a purely random outcome.

The genetic structure of landraces, therefore, may be considered an evolutionary approach to survival and performance under arid and semi-arid conditions (Schulze, 1988). It follows that, during millennia of cultivation under adverse conditions, natural and artificial selection have not been able to identify either an individual genotype possessing a key trait associated with its superior performance, or an individual genotype with a specific architecture of different traits. On the contrary, the combined effects of natural and artificial selection has led to diversity in architecture of genotypes, representing different combinations of traits. These populations can be extremely useful for understanding mechanisms that enhance stability in stress environments, not only from the genetic structure point of view, but also for understanding the adaptive role of given traits. In fact, although variable, landraces grown in environments characterized by a high frequency of stress conditions tend to present a high frequency of a given expression of specific traits.

For example, barley lines extracted from landraces collected from five sites in the Syrian steppe (Table 16.4) were compared with barley lines extracted from landraces collected in Jordan and with a wide range of

TABLE 16.4
Mean of morphological and developmental traits in 1041 modern barley genotypes (unrelated to Syrian or Jordanian landraces) compared with 322 pure lines extracted from Syrian landraces and 232 pure lines from Jordanian landraces

Traits	Modern (n=1041)	Landraces	
		Syrian Arab Republic (n=322)	Jordan (n=232)
1. Early growth vigour	2.5 b	3.2 a	2.4 b
2. Growth habit	2.8 c	4.0 a	3.1 b
3. Cold tolerance	3.0 a	1.3 c	2.3 b
4. Days to heading	117.9 b	121.2 a	116.9 c
5. Grain filling	39.3 a	35.5 c	37.4 b
6. YP (t/ha)	4.398 a	3.293 c	3.947 b
7. YD (t/ha)	0.483 c	0.984 a	0.835 b

Notes: (i) Traits 1, 2, 4, 5 & 6 were scored or measured at Tel Hadya in 1987/88 (504.2 mm rainfall); trait 3 was scored at Bouider in 1987/88 (385.7 mm rainfall); and trait 7 was measured at Bouider in 1988/89 (189 mm rainfall), on 521 modern lines, 92 Syrian landraces, and 86 Jordanian landraces. Early growth vigour (1=good; 5=poor), Growth habit (1=erect; 5=prostrate), Days to heading (days from emergence to awn appearance), Grain filling duration (days between heading and maturity), YP = Yield Potential, YD = Yield under Drought.
(ii) Means followed by the same letter are not significantly ($P<0.05$) different based on t-test for samples of unequal size.

modern (non-landrace) barley genotypes. The Syrian lines showed a higher frequency of genotypes with prostrate or semi-prostrate growth habit, cold tolerance and short grain-filling period, and a lower frequency of genotypes with good growth vigour and early heading. Their average grain yield in unfavourable conditions (at Bouider in 1989) was 0.984 t/ha (ranging from 0.581 to 1.394 t/ha), more than twice the average grain yield of modern genotypes (0.483 t/ha, ranging from crop failure to 1.193 t/ha). The average yield in favourable conditions of the Syrian landraces (3.293 t/ha) was 75 percent of the average yield in favourable conditions of the modern germplasm (4.398 t/ha).

Although this particular set of data is based on one environment only, it confirms the existence of the trade-off between yield in unfavourable conditions and yield in favourable conditions discussed earlier. Landraces collected in Jordan, from sites with milder winters than the Syrian steppe, have a higher frequency of genotypes that have better early growth vigour, more erect habit, less cold tolerance, slightly longer grain-filling period and earlier heading than Syrian landraces. Their average grain yield in unfavourable conditions was only slightly lower (0.835 t/ha) than Syrian landraces, while their average yield in favourable conditions (3.947 t/ha) was in between the Syrian landraces and the modern germplasm. Syrian landraces therefore show a combination of escape (early maturity) and avoidance (prostrate habit and cold tolerance result in good ground cover) mechanisms.

In addition to the high frequency of combinations of escape and avoidance traits, landraces possess another powerful mechanism. They are composed of a number of genotypes with a variable expression for each of these traits. The variability around a mean expression of each character—which already allows a high degree of adaptation—might perhaps be considered as a fine-tuning mechanism to cope with environmental fluctuations. Thus, 321 lines derived from Syrian landraces were classified according to the score for early growth vigour in three classes: good vigour (score <2.5); intermediate (score = 2.5–3.5); and poor vigour (score >3.5). Each class was then classified according to the score for growth habit (erect <2.5; semi-prostrate = 2.5–3.5; prostrate >3.5). No genotypes were found in the good vigour-erect, intermediate vigour-erect, poor vigour-erect, and poor vigour-semi-prostrate classes (Table 16.5).

The groups were compared not only for the two traits used in their classification, but also for days to heading, cold tolerance and length of the grain-filling period. Lines with good early growth vigour tend to be less cold tolerant, earlier and with a longer grain-filling period. This small percentage of genotypes will presumably have a yield advantage in years with slightly milder winter temperatures, absence of late frosts and less severe terminal stress. The highest frequency of genotypes (71.3 percent) combines intermediate early-growth vigour with semi-prostrate or prostrate growth habit. These genotypes are slightly more cold tolerant than the first group, but are slightly later in heading. However, they are better equipped to escape terminal drought because of the shorter grain-filling period. About one-quarter of the genotypes (22.1 percent) have poor early growth vigour but a very prostrate growth habit (growth habit score = 4.2) and a high level of cold tolerance (1.3). Their slightly, although significantly, later heading is not necessarily a negative attribute, mostly because it is compensated for by a very

TABLE 16.5
Frequency of different combinations of early growth vigor (GV), and growth habit (GH), and mean values of cold tolerance (CT), days to heading (DH) and length of the grain filling period (GF) in a sample of 322 lines of barley collected in the dry areas of the Syrian Arab Republic (from same trials as indicated in notes of Table 16.4)

Groups	%	GV	GH	CT	DH	GF
1. Good vigour–Erect	0.0	-	-	-	-	-
2. Good vigour–Semiprostrate	1.2	2.2	3.3	1.6	118.8	37.4
3. Good vigour–Prostrate	5.3	2.4	3.9	1.4	119.8	36.6
4. Intermediate vigour–Erect	0.0	-	-	-	-	-
5. Intermediate vigour–Semiprostrate	6.2	2.9	3.4	1.5	119.7	35.8
6. Intermediate vigour–Prostrate	65.1	3.1	4.0	1.4	121.2	35.4
7. Poor vigour–Erect	0.0	-	-	-	-	-
8. Poor vigour–Semiprostrate	22.1	3.9	4.2	1.3	121.9	35.4
9. Poor vigour–Prostrate	22.1	3.9	4.2	1.3	121.9	35.4
Least Signifigant Difference – $LSD_{0.05}$		0.2	0.1	0.1	0.6	0.7

Notes: All collection sites are included in the Palmyra region, as defined by Weltzien (1988).

short grain-filling period. In an environment characterized by a combination of different abiotic stresses with varying intensity and frequency every year, a population with such architecture of genotypes is probably the best solution to long-term stability (Ceccarelli, Acevedo and Grando, 1991).

The evidence that, at least in the short term, some individual genotypes (pure lines) are able to show the same degree of stability as local heterogeneous populations has been presented earlier (the examples of cvs. Tadmor and Arta). Even so, the use of population buffering in addition to individual buffering offers scope for further increased stability.

In conclusion, the evidence discussed suggests that:

- genetic differences in yield and yield stability under conditions of low winter temperatures and moisture stress are associated with differences, among others, in morphological and developmental traits such as growth habit, cold tolerance, growth vigour and time to flowering. In other types of stress environments and/or in other crops the suite of traits will obviously be different;
- it is the interaction among these, and possibly other traits, that plays a key role in determining the differences in overall performance rather than the expression of any one of them taken in isolation;
- because of the interactions among traits, different combinations of traits are expected to produce the same effect in terms of final yield;
- the role of each individual trait, even within the restricted terms of reference that have been chosen, depends on the frequency, timing and severity of stresses, and on the type of stress; therefore, efforts to identify individual traits causally associated with yield stability under stress are unlikely to be successful;
- in this type of stress environment, 'drought resistance', defined in terms of yield under stress, is a genetic abstraction as much as yield in general;
- analytical breeding to enhance yield stability in stress environments has to consider individual traits as part of an architecture, rather than in isolation; and
- long-term and sustainable improvements of yield stability should probably

be based on population buffering as achievable with mixtures of genotypes representing different, but equally successful, combinations of traits, as occurs in landraces.

16.8 PLOT TECHNIQUES AND EXPERIMENTAL DESIGNS

When genotypes are compared at increasing levels of moisture stress, small variations in soil depth, texture and topography have increasingly large effects on plot-to-plot variability because of associated differences in soil moisture availability. Therefore, it becomes essential to adopt plot techniques and experimental designs that can minimize these effects.

Various plot techniques to increase the efficiency of direct selection in the presence of abiotic stresses have been discussed extensively in Chapter 3 of this volume.

16.9 DECENTRALIZED-PARTICIPATORY SELECTION

The term 'decentralized selection' was first used by Simmonds (1984) and defined as selection in the target environment(s). Decentralized selection becomes selection for specific adaptation when the selection criterion is the performance in specific environments rather than the mean performance across environments. Selection for mean performance across a number of environments (years and locations) tends to exclude breeding material that performs very well in the lowest yielding years or locations but not particularly well in the highest yielding years or locations, unless data are standardized. On the contrary, selection for the highest yielding breeding material in specific locations or areas will automatically include breeding material performing well across all locations. In other words, selection for specific spatial adaptation will not exclude breeding material with wide spatial adaptation, while selection for wide adaptation tends to eliminate breeding material with specific adaptation.

Decentralized selection is different from decentralized testing, which is a common feature of breeding programmes and takes place, usually in the form of multilocation trials and on-farm trials, after a number of cycles of selection in one or few environments (usually with high levels of inputs).

Decentralized breeding is a powerful means to adapt crops to the physical environment. However, to exploit fully the potential gains from specific adaptation to low-input conditions, breeding must be decentralized from research stations to farmers' fields. Although decentralization and farmer participation are unrelated concepts, decentralization to farmers' fields almost inevitably leads to the participation of farmers in the selection process (Ceccarelli and Grando, 2002).

16.10 DECENTRALIZED-PARTICIPATORY PLANT BREEDING

The implementation of decentralized-participatory plant breeding (PPB) programmes started in 1997 with the aim of developing an alternative way of conducting plant breeding that is much more efficient and much quicker in bringing new varieties to farmers, and ensures that the new varieties are adapted to farmers' specific environments and end-uses.

The emphasis of the programme has been on dry areas, even though the approach can also be beneficial to high-rainfall environments.

The programme, which has been described in detail by Ceccarelli and Grando (2007) and by Ceccarelli, Grando and Baum, (2007), is based on the following concepts:

- the trials are grown in farmers' fields using the host farmer's agronomic practices;
- selection is conducted by farmers in farmers' fields, so that farmers are the key decision-makers; and
- the traditional linear sequence of Scientist → Extension → Farmers is replaced by a team approach, with Scientists, Extension Staff and Farmers participating in all major steps of variety development (see Figure 9.1).

In a conventional breeding programme, the most promising lines are released as varieties, their seed is produced under controlled conditions (certified seed) and only then can farmers decide whether to adopt them or not. In many developing countries the process results in many varieties being released but only a small fraction being adopted. The major consequence of the PPB concept is that the process transforms the delivery phase of a plant breeding programme from being supply driven to being demand driven.

Under PPB, it is the initial farmers' preference that drives the decision of which variety to release. As a consequence, adoption rates are higher, and risks are minimized, as intimate knowledge of varietal performance is gained as part of the selection process. Last, but not least, the public investment in seed production is nearly always paid off by farmers' adoption.

The programme started in the Syrian Arab Republic in 1996 and was expanded to Algeria, Egypt, Eritrea, Islamic Republic of Iran, Jordan, Morocco, Tunisia and Yemen, using the same bulk-pedigree method described earlier. Four types of impact can be observed, considered below.

Variety development

New varieties were spontaneously disseminated from farmer to farmer as early as three years after starting the programme. In the Syrian Arab Republic, several thousand hectares are planted with two varieties, and 12 varieties have been adopted by farmers and are under seed multiplication (Table 16.6). Varieties are adopted both in dry areas and in wetter areas in a much shorter time than in a conventional breeding programme. It also confirms the importance of landraces (Tadmor, Arta, SLB and JLB lines, Zanbaka, A. Abiad and A. Aswad) as well as *H. spontaneum* when farmers' opinion becomes part of the breeding process.

TABLE 16.6
Varieties adopted from the PPB programme by farmers in the Syrian Arab Republic

Pedigree	Name	Location	Rainfall
H.spont.41-1/Tadmor	Raqqa-1	Bylounan	212.4
Arta//H.spont.41-5/Tadmor	Raqqa-2	Bylounan	212.4
Zanbaka/JLB37-064	Karim	Bylounan	212.4
Tadmor/3/Moroc9-75/ArabiAswad//H.spont.41-4	Akram	Bylounan	212.4
Mo.B1337/WI2291//Moroc9-75/3/SLB31-24	Suran-1	Suran	383.7
ChiCm/An57//Albert/3/Alger/Ceres.362-1-1/4/Arta	Suran-2	Suran	383.7
ER/Apm//Lignee131/3/Lignee131/ArabiAbiad/4/Arta	Suran-3	Suran	383.7
Hml-02/5/..Alger/Ceres362-1-1/4/Hml	Nawair-1	Suran	383.7
Hml-02/5/..Giza 134-2L/6/Tadmor	Nawair-2	Suran	383.7
SLB03-10/Zanbaka	Yazem	J. Aswad	226.4
Tadmor//Roho/Mazurka/3/Tadmor	Salam	J. Aswad	226.4
ArabiAswad/WI2269/3/ArabiAbiad/WI2291//Tadmor /4/Akrash//WI2291/WI2269	Ethiad	J. Aswad	226.4

Note. Rainfall is annual rainfall in mm, average of the period 2000–2005.

Institutional

In several countries, PPB has generated considerable change in the attitude of policy-makers and scientists towards the benefits of participatory research, and generated changes in national breeding programmes.

Farmers' skills and empowerment

The cyclic nature of the PPB programmes has considerably enriched farmers' knowledge, improved their negotiation capability, and enhanced their self esteem. By the same token, scientists (breeders) have been enriched by the farmers' indigenous knowledge of the crops they grow and the environments in which they grow them.

Enhancement of biodiversity

Different varieties have been selected in different areas within the same country, and even within the same location (as shown in Table 16.6) in response to different environmental constraints and users' needs.

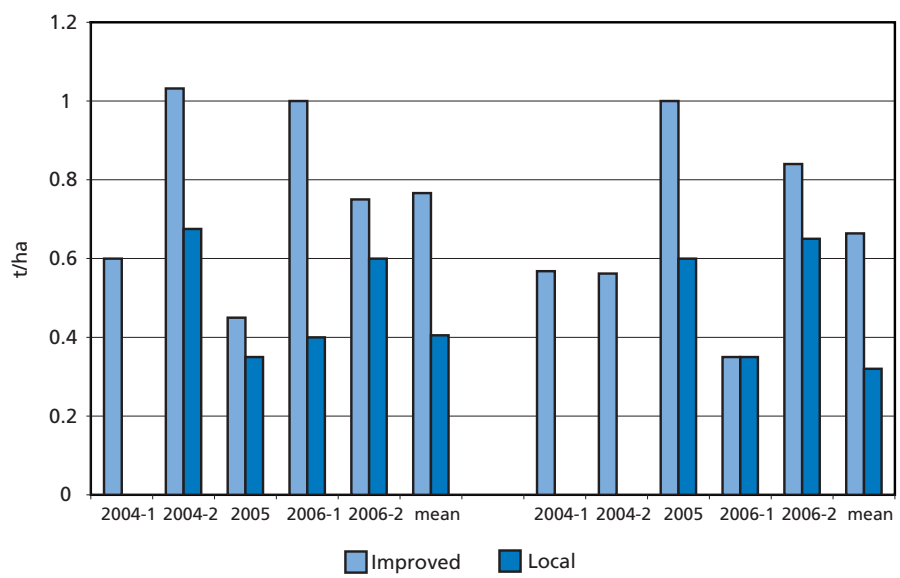
16.11 DROUGHT-RESISTANT LINES

PPB was not specifically designed to breed for drought tolerance, but rather to adapt the crops to a number of environmental and agronomic conditions and to farmer preferences. These also include situations where drought stress occurs frequently and can be very severe. Therefore it is not surprising that the PPB programme has produced the breeding material with the highest level of drought tolerance.

In this section we will give two examples of lines specifically adapted to dry areas.

The first example refers to lines selected in the Syrian Arab Republic in 2000, when the total rainfall in most areas of the country was

FIGURE 16.3
Grain yield (t/ha) of two lines with improved drought resistance in comparison with the local landrace tested in 2004, 2005, and in 2006 in rainfed location in the Syrian Arab Republic receiving less than 200 mm of total rainfall



Line 1=H.spont.41-1/Tadmor//SLB45-090/H.spont.41-2/3/H.spont.41-1/Tadmor//H.spont.41-1/Tadmor
Line 2=Arta/3/Arar/H.spont.19-15//Hml

below average and crop yields were severely affected. In some areas, the rainfall was so low that the crop did not even germinate; in many others the crop failed to produce grain. The PPB trials, planted in eight farmers' fields in the Syrian Arab Republic, were affected by different intensities of drought. At one extreme, the rainfall was only 50 mm in the entire season and no germination occurred. At the other extreme, the rainfall was 252 mm rainfall and average grain yield was 1.8 t/ha (ranging from 1.0 to 3.2 t/ha). The driest sites, where some new barley entries were able to produce some grain or some biomass, received between 87 and 130 mm. Average grain and biomass yield were very low but some lines were able to produce between 0.3 and 0.5 t/ha of grain and between 0.5 and 3.0 t/ha of biomass yield (Ceccarelli *et al.*, 2004).

Two of these lines were tested by farmers on large areas (5–20 ha) in 2004, 2005 and 2006, which were all very dry (Figure 16.3). In comparison with the local landrace, which itself is considered to be drought resistant, the two lines showed an average yield advantage of 44 percent, ranging from 9 percent to 67 percent. This includes one case in which the local landrace failed, and one of the improved lines yielded 0.5 t/ha. Both lines are derived from crosses with a pure line of *H. spontaneum* (the wild progenitor of cultivated barley), an indication that some *H. spontaneum* lines can contribute significantly to enhance the drought resistance of cultivated barley.

TABLE 16.7
Rainfall (mm) and average yield (in parenthesis in t/ha) in five locations in the dry areas of the Syrian Arab Republic during the three years 2003–2005

Location	No. of lines	2003	2004	2005	Mean annual rainfall 2000–2005 (mm)
Bylounan	8	187 (1.249)	217.4 (0.804)	196 (0.604)	212.4
J. Aswad	6	215 (1.152)	245.3 (0.808)	238 (0.853)	226.4
Melabya	10	275.9 (1.496)	176 (0.432)	187.5 (0.466)	186.5
Siebatt	12	308 (1.406)	319 (0.478)	263 (1.027)	248.8
Al Bab	10	408 (1.355)	296 (0.422)	289.5 (1.084)	307.5

TABLE 16.8
Grain yield (as percentage of the local check) of the highest yielding lines during 2003, 2004 and 2005 in five dry locations in the northern Syrian Arab Republic receiving between 186 and 308 mm total annual rainfall

Location	Line	2003	2004	2005	Mean
Bylounan	Arta/SLB22-74	1.109	1.030	1.065	1.068
Bylounan	ArabiAbiad/Arar//H.spont.41Arta/…..	1.087	1.100	0.993	1.060
Bylounan	Arta//H.spont.41-5/Tadmor/3/SLB05-096	1.098	1.102	0.982	1.060
J. Aswad	SLB28-53/SLB21-81	1.030	1.121	0.967	1.039
Melabya	Roho/4/Zanbaka/3/ER/Apm//Lignee131	1.151	1.045	1.071	1.089
Melabya	ArabiAbiad/Arar//H.spont.41Arta/…..	0.957	1.038	1.101	1.032
Siebatt	SLB21-81/SLB22-74	1.216	1.085	1.023	1.108
Siebatt	Anadolu86/Sara-02//Zanbaka	1.186	0.993	1.128	1.102
Siebatt	Zanbaka/SLB21-81	1.180	1.038	1.036	1.084
Siebatt	Sara-01/Sara	1.433	1.009	0.998	1.147
Al Bab	ChiCm/An57//Albert/3/Alger/Ceres.362	1.323	1.535	1.207	1.355
Al Bab	SLB28-53/SLB21-81	1.282	1.472	1.300	1.352

The second example derives from trials conducted in dry locations in the northern Syrian Arab Republic during the period 2003–2005. Two locations (Bylounan and Melabya) represent some of the driest areas in the Syrian Arab Republic, where barley is the only possible rainfed crop; J. Aswad and Siebatt are in slightly wetter areas, while Al Bab is a location characterized by colder winters than the other four. In the two driest locations, rainfall varied from 176 mm to 245.3 mm total annual rainfall and average grain yield from 0.432 to 1.496 t/ha during the testing period. In the two wetter locations, rainfall varied from 215 to 319 mm total annual rainfall and average grain yield from 0.478 to 1.406 t/ha, while Al Bab was the wettest of the five locations but not the highest yielding because of the low temperatures in winter (Table 16.7).

In the five locations, we tested between 6 and 12 lines (including the checks) representing the result of two cycles of decentralized participatory selection starting from a common set of 165 lines. The yield of the best lines is shown in Table 16.8, expressed as a percentage of the local check.

At the two driest sites, Bylounan and Melabya, five lines outyielded the local check on average over 3 years by between 3.2 percent and nearly 9 percent, but only three lines were consistently superior to the local check in each of the three years. In the two wetter locations, five lines outyielded the local check by between 7.5 percent and 14.7 percent, but only the two lines in J. Aswad and two of the four lines in Siebatt consistently outyielded the local check. In Al Bab, two lines consistently outyielded the local check by slightly more than 35 percent; two lines (ArabiAbiad/Arar//H.spont.41 and SLB28-53/SLB21-81) were among the highest yielding lines in two locations (Bylounan and Melabya the first and Al Bab and J. Aswad the second). As these lines are the product of one cycle of selection, further progress is expected with additional cycles of recombination and selection.

16.12 CONCLUDING REMARKS

The objective of this chapter has been to discuss what plant breeders can do when the target environment of their breeding programme is characterized by chronic low yields due to numerous factors, such as climatic, nutritional and abiotic stresses. The data are mostly derived from barley and from one type of dry area (dry Mediterranean with cold winters and hot summers, and crops grown on current rainfall). However, the paper illustrates some general concepts that, with some modifications, could be useful in other crops and in other types of dry area.

The first concept is that in these environments, climatic, nutritional and biotic stresses usually occur together (though not necessarily all of them all of the time); and, so far, there is little substitute for actually exposing the breeding material to a real field situation. Although little practiced, the idea is not new. Nearly forty years ago Hurd (1971) published a paper with the title: Can We Breed for Drought Resistance? The first sentence of the paper was "My answer to the above very pertinent question is a confident and optimistic 'Yes'". He concluded: "One method is to grow large populations in early generations under typical dry growing conditions." Twenty years later, Bramel-Cox *et al*., (1991) recognized that the key to increased production with fewer external inputs would be through a re-evaluation of the identification and use of selection and testing environments.

Although this concept is obvious to

many and not new, selection for stress environments is still seldom done in the target environments and it still a highly controversial issue, as it is the relationship between high yield under optimum conditions and high yield under abiotic stress conditions (see, for example, Chapter 18 in this volume). This may not always be necessarily a deliberate choice of one breeding strategy or another, but is simply due to the distance of suitable selection sites from main cities, with all the associated inconveniences. We hypothesize that in these cases an interesting solution may be offered by farmers' participation in breeding (Ceccarelli and Grando, 1997; Ceccarelli, Grando and Baum, 2007). Conducting selection in farmer's fields has the advantage of exploiting genetic differences under farm conditions, with the additional advantage of making use of the farmer's knowledge of the crop.

The second concept is the use of germplasm usually ignored by most plant breeders, such as landraces and wild relatives. This approach is a direct consequence of choosing to work in the target environment and has led to the development of a number of barley cultivars, now grown in a number of farmers' fields in the central and northern Syrian Arab Republic and in environments considered too difficult and therefore beyond the plant breeder's domain.

The third concept is that in dry areas, every effort should be made to control environmental variability in trial and nurseries evaluation. When working at stress sites, the breeder should forget the typical research-station style of work. The methodology, experiment designs and plot techniques used in the very homogeneous environment of the experiment station are not suitable; in fact, when the conclusion is reached that progress cannot be made in a stress environment, it is probably for that reason.

The main conclusion of this paper is that breeding for stress environments is possible, provided it is conducted with strategies and methodologies that little have in common with those used in breeding for favourable environments. Adaptation over time can be improved by breeding for specific adaptation to a given type of stress environment. This can be achieved by taking advantage of the temporal variability of stress environments, which permits exposure of the same breeding material to variable combinations of stresses over a (relatively) short period and to accumulate favourable alleles at the several loci involved in drought resistance through successive cycles of recombination and selection. We are aware that this is fundamentally different from the modern trend of plant breeding towards broad adaptation over space. The difference represents the contrasting interests of farmers and seed companies. Farmers are interested in cultivars that are consistently superior on their farm, regardless of how they perform at other locations or in other countries. Seed companies, however, want to market as much seed of as few cultivars as possible. Breeders have been breeding, perhaps unconsciously, more for seed companies and for their personal prestige than for farmers. The two objectives coincide when selection and target environments are similar, but this approach has by-passed millions of small farmers in difficult environments.

Recent advances in plant genomics have enabled one to dissect various molecular mechanisms (signal transduction pathways) involved in drought, cold and salt stress tolerance and in identifying various genes involved in such stress tolerance. Information generated in genomics should

be integrated into practical plant breeding. Various genes identified, in both model plants and crop plants, could be used in future for developing stress-tolerant plants through either marker-assisted selection or direct gene transfer.

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CHAPTER 17

Breeding for quantitative variables Part 4: Breeding for nutritional quality traits

Yusuf Genc, Julia M. Humphries, Graham H. Lyons and Robin D. Graham

17.1 INTRODUCTION

Malnutrition is the most important cause of mortality in the global human population. More than a quarter of children less than five years old suffer from protein-energy malnutrition, as determined by rates of stunting and underweight. Of these, 70 percent are in Asia, 26 percent in Africa and 4 percent in Latin America. Stunting in resource-poor populations is usually associated with reduced mental development (Stephenson, Latham and Otteson, 2000).

Dietary deficiencies of the micronutrients iron (Fe), zinc (Zn), vitamin A (in the form of pro-vitamin A carotenoids), selenium (Se) and iodine (I) are widespread globally, affecting well over half of the world's population, and often occur concurrently (WHO, 2003). These deficiencies increase the risk of severe disease in approximately 40 percent of the world's population (Graham, Welch and Bouis, 2001). Se, Fe, Zn and vitamins A, B and C have immunomodulating functions and thus influence the susceptibility of a host to infectious diseases and their courses and outcomes (Bhaskaram, 2002; Failla, 2003).

Most of the Fe in the body occurs in combination with proteins as the oxygen-carrying pigments haemoglobin in red blood cells and myoglobin in muscle cells. Deficiency results in reductions in haemoglobin (anaemia) and tissue Fe (myoglobin and Fe-containing enzymes), and in lethargy (Jones, 1997).

Zn is a component of over three hundred enzymes, involved in carbohydrate metabolism, DNA synthesis, protein synthesis and digestion, and bone metabolism. Deficiency can result in reduced growth rate, skin lesions and increased susceptibility to infection (Jones, 1997).

Vitamin A deficiency is a major cause of blindness, growth retardation and increased susceptibility to infection. It commonly occurs in association with protein and Zn deficiency (Wahlqvist, 1997). This chapter will deal with the plant precursors to vitamin A: carotenoids such as β-carotene, and the non-provitamin A carotenoids, including lutein and zeaxanthin. Carotenoids are responsible for many of the orange, red and yellow colours seen in plants and animals. A small proportion of the over 600 named carotenoids are precursors to vitamin A, and are essential for the prevention of vitamin A deficiency. β-carotene has the highest provitamin A activity. Carotenoids that are not precursors to vitamin A also have an important role in health and nutrition as antioxidants and in the maintenance of sight, and those most commonly found in staple foods are lutein and zeaxanthin. Lutein and zeaxanthin are abundant in maize, and lutein is the dominant carotenoid in both bread and pasta wheat.

Se is an integral component of at least three systems required for normal cell metabolism, and has antioxidant, anti-cancer and anti-viral effects (Arthur, 1999). I is involved in growth, development and metabolic regulation, through its role as a component of thyroid hormones (Hetzel and Pandav, 1996). Moreover, interactions between Se and I are important in the body. Both micronutrients are required for thyroid hormone synthesis, activation and metabolism, and the thyroid gland has the highest Se and I concentrations of all organs (Kohrle, 1999).

HarvestPlus is a Biofortification Global Challenge Program of the Consultative Group on International Agricultural Research (CGIAR). It is coordinated by the International Centre for Tropical Agriculture (CIAT) and the International Food Policy Research Institute (IFPRI). Genetic bio-fortification is a strategy of

breeding staple crops such as rice, wheat, barley, maize, cassava, potatoes and beans with the ability to fortify themselves with micronutrients. It offers a sustainable, cost-effective alternative to other strategies such as individual supplementation and fertilization, which is more likely to reach those most in need and has the added advantage of requiring no change in current consumer behaviour to be effective (Graham, Welch and Bouis, 2001). Once a one-off investment is made to breed bio-fortified seed, recurrent costs are low and germplasm can be shared globally. Bio-fortification, commercial fortification and supplementation are complementary strategies for reaching malnourished populations. Furthermore, bio-fortification can increase farm productivity as certain micronutrients, such as Zn and Se, that improve human nutrition can help plants resist diseases and other environmental stresses (HarvestPlus, 2007).

Breeding criteria for micronutrient-enriched staple food crops have been reviewed recently (Welch and Graham, 2004). These criteria include (i) maintaining crop productivity, (ii) evidence for stability of micronutrient enrichment traits across various edaphic and climatic zones, (iii) demonstration of significant effects of enriched micronutrients on human health, (iv) demonstration of bio-availability of enriched micronutrients for human nutrition, and (v) consumer acceptance.

In this chapter, we will focus on genetic potential, genotype × environment interactions, screening protocols, breeding strategies for enhancing grain micronutrient accumulation. Physiological and molecular mechanisms of uptake, translocation and deposition of micronutrients in the grains or other edible parts of major staple food crops such as wheat, rice, maize, beans and cassava, which are consumed by billions of people in resource-poor nations will also be discussed. Sufficient genotypic variation in the trait to be selected is necessary for conventional breeding to be feasible, so we will discuss this as a first step, with reference to the five key micronutrients. Breeding principles discussed in this chapter are applicable to both traditional and participatory plant breeding.

17.2 GENOTYPIC VARIATION OF MICRONUTRIENT CONCENTRATION IN STAPLE FOOD CROPS

17.2.1 Fe & Zn

Over the last decade, there have been considerable efforts in several international research centres such as the International Maize and Wheat Improvement Centre (CIMMYT) in Mexico and the International Rice Research Institute (IRRI) in the Philippines, to identify wheat and rice germplasm with high Fe and Zn concentration, which has also been the subject of several reviews (Graham *et al.*, 1999; Rengel, Batten and Crowley, 1999; Cakmak *et al.*, 2000).

Wheat

At CIMMYT, in one study, 170 wheat lines selected out of 550 initially screened lines were grown in a replicated trial (Ortiz-Monasterio and Graham, 2000). This study identified three promising sources of high grain Fe and Zn concentration: wild species, landraces and breeding lines. Fe concentration was in the range of 25 to 56 mg/kg dry weight (DW), while Zn concentration varied from 25 to 65 mg/kg DW. In a second study, a group of 154 lines from the breeding programme were grown together. Lines were identified with up to 73 mg Fe/kg DW and 92 mg Zn/kg DW. Our group at the University of Adelaide also observed significant variation in grain Zn and Fe concentrations in commercial cultivars and advanced breeding lines

TABLE 17.1
Zn and Fe concentrations (mg/kg DW) in grains of durum and bread wheat genotypes grown in standard potting mix with all nutrients supplied adequately in a glasshouse

	No. of entries	Fe		Zn	
		Mean conc. (SD)	Range	Mean conc. (SD)	Range
Modern bread wheat (*T. aestivum*)	25	36 (6)	27–53	39 (8)	25–53
Synthetic hexaploid wheat (*T. aestivum*)	36	41 (8)	32–67	41 (9)	28–66
Durum wheat (*T. dicoccon*)	24	42 (7)	29–56	51 (6)	39–62
(*T turgidum*)	191	33 (8)	17–62	30 (12)	12–81

Note: Standard deviation, SD, is given in parentheses.
Source: Genc *et al*., unpublished.

TABLE 17.2
Zn and Fe concentrations (mg/kg DW) in grains of bread wheat genotypes in field trials in Australia

Location and year	No of entries	Fe		Zn	
		Mean conc. (SD)	Range	Mean conc. (SD)	Range
Birchip-2000	28	37 (3)	31–41	16 (2)	12–19
Birchip-1999	42	38 (2)	32–42	25 (3)	20–31
Birchip-1998	39	42 (4)	36–55	23 (2)	19–31
Horsham-1998	30	33 (3)	27–40	16 (1)	13–19
Bute-1997	42	32 (3)	27–38	18 (2)	15–21
Lameroo-1996	35	33 (3)	27–39	20 (2)	15–24

Note: Standard deviation, SD, is given in parentheses
Source: Graham *et al.,* unpublished.

of wheat in glasshouse and field trials conducted over a number of years in Australia (Tables 17.1 and 17.2). In general, grain Zn and Fe concentrations were higher in the glasshouse than in field studies, which can be attributed to better growing conditions (well-watered and fertilized) in the glasshouse than in the field. In the field studies, grain Zn concentration was in the range of 12 to 31 mg/kg DW, a narrower range than that found at CIMMYT. This narrower range and also lower values (<15 mg/kg DW) in grain Zn concentration are indicative of Zn deficiency in the field. Grain Fe concentration varied from 27 to 55 mg/kg DW. Recently, a large-scale screening by Cakmak *et al.* (2004) has identified wild wheat accessions with even higher Fe and Zn concentrations than those reported previously. In this comprehensive study of 825 accessions, including wild emmer wheat (*Triticum turgidum* subsp. *dicoccoides*), grain concentrations were 14 to 190 mg/kg DW and 15 to 109 mg/kg DW for Zn and Fe, respectively. In this study, no yield data were reported, thus we do not know whether high concentrations are associated with low yield. In the meantime, despite lower Fe and Zn concentrations than in wild species, more screening is needed of elite germplasm (modern wheat genotypes and advanced breeding lines) for high Fe and Zn concentration in the grain, as they have already improved agronomic performance (Graham *et al.*, 1999).

Rice

There also exists considerable genotypic variation for grain Zn and Fe concentration in rice. Researchers at IRRI and the

University of Adelaide (Australia) have evaluated a large set of brown rice varieties (1138), including breeding lines and wild rice and its derivates, and observed a wide range in Fe (6–24 mg/kg DW) and Zn (14–58 mg/kg DW) concentrations in the grain (Gregorio *et al.,* 2000). Some of these high-Fe and-Zn varieties were further tested alongside the two most popular cultivars in Asia, IR36 and IR64, in the same soil and year. The traditional variety, Jalmagna, had much higher grain Fe and Zn concentration than high-yielding IR36 (22 vs 12 mg Fe/kg DW; 32 vs 21 mg Zn/kg DW in Jalmagna and IR36, respectively). Moreover, aromatic rices are often reported to have higher grain Fe and Zn concentration than non-aromatic rices (Graham, Senadhira and Ortiz-Monasterio, 1997; Gregorio *et al.,* 2000).

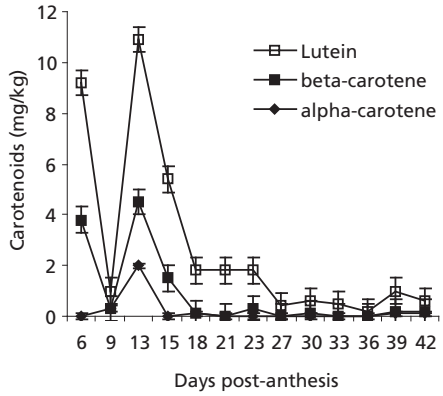
There is some evidence that breeding for either high Fe or Zn may also result in higher concentrations of other nutrients, as there is occasionally a significant positive correlation between Fe and Zn concentrations in the wheat and rice grain (Ortiz-Monasterio and Graham, 2000; Graham, Senadhira and Ortiz-Monesterio, 1997; Genc *et al.,* unpublished). This is also evident in a recent study by Vasconcelos *et al.* (2003), who introduced a soybean *ferritin* gene into *indica* rice, and found much higher Fe and Zn concentrations in the grains of transgenic plants compared with non-transgenic plants. However, as no seed weight data were provided, we do not know whether these high concentrations were associated with small seed size (concentration effect). Nevertheless, our calculations of their data established a significant positive correlation between grain Fe and Zn ($r^2 = 0.54$).

17.2.2 Vitamin A

The potential for finding genetic variation that can form the basis for breeding crops with increased carotenoid concentrations is great, given that all photosynthetic organisms have substantial concentrations of these compounds. However, in many staple crops it is necessary for the plant to store carotenoids in non-photosynthetic tissues, such as the tuber of the sweet potato, or in tissues that no longer have a photosynthetic capacity when harvested, as in wheat and maize. It is possible that the consumed portion of the crop that once had photosynthetic capacity may retain the carotenoids accumulated during the photosynthetic period post-degradation of the chlorophyll. However, in root crops, carotenoids must accumulate in non-photosynthetic tissues, and therefore need to be transported there from other photosynthetic tissues, or synthesized *de novo*.

A report by Graham and Rosser (2000) compared the synthesis patterns during maturation of both lutein and β-carotene in bread and durum wheat varieties (Figure 17.1). These results concur with an earlier report of Lacroix and Lier (1975), and indicate that a potential benefit may be gained by harvesting wheat at the immature (green) stage. However, appropriate storage and preservation methods are necessary in order for immature wheat to be stored for any period of time without spoilage. Such a method has been used for centuries in Middle Eastern countries, where wheat is harvested green and dry roasted to produce a product called *freekeh*. Substantial amounts of both β-carotene and lutein can be conserved from the photosynthetic stage in the roasted product by this method (Humphries and Khachik, 2003). This method of storage may be a valuable starting point for adoption into local cultures for

FIGURE 17.1
Time trend of synthesis and metabolism of various carotenoids in the caryopsis of wheat (var. Krichauff) from anthesis to maturity



Source: Graham and Rosser, 2000. Reprinted with permission from the UNU Food and Nutrition Bulletin (2000) 21: 404–409.

preservation of carotenoids present in immature wheat.

Selection against highly pigmented varieties in several staple foods has led to very little variation for this trait in modern cultivars. However, germplasm banks where landraces and old varieties are stored hold the key to retrieving genetic variation. These valuable resources can be used as a source of variation for the introduction of desirable traits back into commercially profitable and locally grown varieties, for nutritional benefit. Reports from screening of genetic resources obtained from germplasm banks indicate that many of the older varieties have substantial variation for carotenoid concentration.

Maize

White maize was previously highly desired for reasons of cleanliness and apparent purity, while maize varieties with high concentrations of pigmentation were used as stock feed. While people suffered from vitamin A deficiency, their stock remained healthy due to consumption of yellow maize (Brunsen and Quackenbush, 1962). Collaboration between nutritionists and agriculturalists resulted in the production of high-β-carotene maize adapted to local conditions, resulting in a reduction in vitamin A deficiency-associated diseases.

There have been several reports of genetic variation for carotenoids in maize. One of the first was that of Brunsen and Quackenbush (1962), who showed that the total concentration of carotenoids varied significantly between high- and low-carotene inbred lines, with an even greater variation between low and high for provitamin A carotenoids. Blessin *et al*. (1963) reported a range of 0.9 to 4.1 mg/kg for carotenes, and 18.6 to 48 mg/kg for xanthophylls in 39 maize inbreds. In the same year, Quackenbush *et al*. (1963) observed concentrations of up to 7.3 mg/kg carotenes, and a range of 2 to 33 mg/kg lutein in 125 inbred lines. The value of extensive screening for high-accumulation varieties was indicated in the report of Egesel (1997), who found a range of just 0.13 to 2.9 mg/kg β-carotene in 200 maize families. More recently, 16 yellow seeded maize lines were reported to have 143 to 278 mg/kg carotenoids (Maziya-Dixon *et al*., 2000). This study measured total carotenoid concentrations rather than defining provitamin and non-provitamin carotenoids, and although valuable for calculation of total carotenoid intake, gives no idea of the provitamin A potential of the cultivars.

Wheat

Wheat is another staple food that has been subjected to selection for colour, though with different outcomes depending on the end use, either bread or pasta. Bread wheat varieties (*Triticum aestivum*) have been

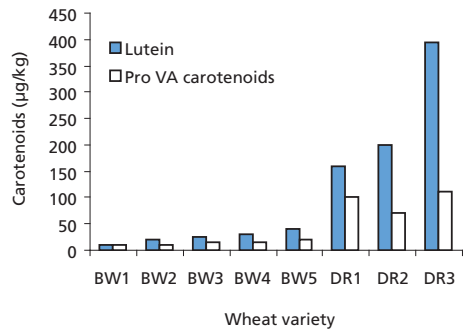
the subject of selection against pigmentation, though ironically, despite breeding efforts, it was still deemed necessary to use chemical bleaching rather than plant breeding alone to achieve the bright white colour demanded by bread wheat consumers. The process of bleaching has now been discontinued. However, the generations of selection against pigmentation has resulted in current cultivars containing very low concentrations of carotenoids. In Australia, there is an exception, a South Australian wheat by the name of Krichauff, which has relatively high concentrations of carotenoids in comparison with other current cultivars. It is also possible that the wheat used to make tortillas in South America may have substantial carotenoid concentrations that could be exploited for nutritional gain.

Matus-Cadiz *et al.* (2003) reported that the lutein concentration of 79 diverse spring wheat varieties from Australia and Canada ranged between 1.8 and 3.7 mg/kg, and also reported genotype × environment (G×E) effects that will be discussed in the G×E section of this chapter. An extensive survey of bread wheat varieties (Humphries *et al.*, 2004) revealed considerable genetic variation for both provitamin and non-provitamin A carotenoid concentrations in germplasm from the CIMMYT germplasm bank. Results from the range of concentrations obtained from bread wheat combined with those for durum wheat varieties are given in Figure 17.2.

Alternatively, the importance of colour in durum wheat (*Triticum durum*) used for pasta, has led to extensive studies into carotenoid concentrations. Several of these studies are presented below, and together with other reports not included here, indicate that despite the apparent potential for a source of high concentrations of carotenoids in different cultivars, concentrations of provitamin A carotenoids are usually low. It appears that selection for the colour provided by lutein has led to varieties with an abundance of the enzymes responsible for hydroxylation of the provitamin A carotenoids, and consequently the durum wheats are not thought to be a useful source of genetic variation for increased provitamin A carotenoids.

Some of the first reports of the dominance of non-provitamin A carotenoids in durum wheat were published in 1935 (Markley and Bailey, 1935a, 1935b), and later it was confirmed that only a small proportion of carotenes were present in comparison to lutein (Munsey, 1938). This was followed up by Zechmeister and Cholnoky (1940) and Lepage and Sims (1968), who reported lutein ester concentration and no provitamin A carotenoids. A more recent evaluation of the carotenoid composition of durum wheat (Hentschel *et al.*, 2002) revealed a range of lutein concentrations from 1.5 to 4 mg/kg, and no carotenes.

FIGURE 17.2
Carotenoid concentrations of five bread wheat varieties (BW) and three durum wheat varieties (DR), representing the range of values found in collections from the germplasm banks in CIMMYT and ICARDA



Source: Graham and Rosser, 2000. Reprinted with permission from the UNU Food and Nutrition Bulletin (2000) 21: 404–409.

Crosses with barley

Tritordeum lines, which are a cross between the wild barley species *Hordeum chilense* and diploid, tetraploid and hexaploid wheat have consistently shown higher concentrations of carotenoids than wheat (Alvarez, Urbano and Martin, 1994), and are considered a useful source for increasing the carotenoid concentration of durum wheat. However, using this cross to increase concentrations is dependent on interactions between the genetics of the parents, and the final concentrations cannot be reliably predicted.

Ancient wheat

Einkorn (*T. monococcum*), an ancient diploid wheat, has been reported to have yellow coloration (D'Egidio, Nardi and Vallega, 1993; Abdel-Aal, Hucl and Sosulski, 1995; Borghi *et al*., 1996). When compared with other ancient wheat varieties, spelt (*T. aestivum* subsp. *spelta*), emmer (*T. turgidum* subsp. *dicoccum*), Kamut (*T. turgidum* subsp. *turanicum*) and Khorasan (*T. turgidum* subsp. *turanicum*) einkorn lines generally had higher concentrations of lutein (mean 8.1 ± 0.26 µg/g) (Abdel-Aal *et al*., 2002).

Cassava

The carotenoid concentration of cassava roots has been closely correlated to the intensity of the root colour. However, within groups of the same tuber colour, genotypic variation has also been reported, from 6 to 24 mg/kg fresh weight (FW) (Chavez *et al*., 2000). This variation within colour types necessitates individual analyses to determine individual concentrations, and colour alone cannot be relied upon to give accurate estimations of concentrations.

One of the largest reported analyses of cassava for carotenoid concentration was conducted by Iglesias *et al.* (1997), who screened a total of 632 accessions from the CIAT germplasm bank collection of 5500. The distribution of concentrations ranged from 1 to 24 mg β-carotene/kg FW. Those varieties with the deepest coloration towards orange had the greatest concentration of carotenoids, which is consistent with the report of Chavez *et al*., (2000).

The variability for carotene concentrations in cassava has been reported for accessions obtained from germplasm banks in India (Moorthy *et al*., 1990) and Brazil (Ortega-Florez, 1991). The highest concentrations were below 8 mg of β-carotene equivalents/kg FW, which is one-third the highest concentration reported by Iglesias *et al*. 1997. However, the potential for rapidly increasing carotene concentrations using recurrent selection was reported by Jos *et al*. (1990). Using this method, it is possible to increase the concentration by three times, from 4.2 to 13.8 mg/kg FW after 2 cycles of selection and recombination (Jos *et al*., 1990). It is therefore possible, in theory, to obtain concentrations of β-carotene up to 72 mg β-carotene/kg FW.

17.2.3 Se & I

To address dietary Se deficiency, agronomists and plant breeders have adopted complementary strategies to develop crops with higher Se content. The first is an agronomic (fertilizer) approach, discussed elsewhere (Lyons *et al.*, 2003, 2004; Broadley *et al.*, 2006). The second strategy is to develop varieties with improved Se accumulation and tolerance traits by either conventional breeding or genetic modification. To implement this approach, a comprehensive characterization of the interactions between Se and sulphur nutrition was conducted in *Arabidopsis* (White *et al.*, 2004). If sufficient genotypic variation exists in Se accumulation within a crop species, and if this variation is heritable, conventional plant breeding could

provide an alternative to agronomic biofortification and thus minimize the need for Se fertilizers (Broadley *et al.*, 2006).

Few data have been published on varietal differences for Se accumulation for most crop species. However, in *Lycopersicon* (tomatoes and related plants), four-fold differences in shoot Se accumulation have been found (Pezzarossa *et al.*, 1999), and in *Brassica* (broccoli) a significant genotype effect for Se concentration in heads was found in hybrids, but not inbreds. However, the effect of environment was around ten times stronger than that for genotype (Farnham *et al.*, 2007).

Wheat

In surveys and trials conducted by our group, involving diverse wheat germplasm and a total of eleven datasets in South Australia and Mexico, grain Se concentrations were in the range of 5 to 720 µg/kg DW, but much of this variation was associated with spatial variation in soil-available Se. South Australian soils are renowned for their microspatial variability, which makes detection of genotypic differences in grain Se density difficult. No significant genotypic variation in grain Se density among modern commercial bread or durum wheat varieties was detected in this study (Lyons *et al.*, 2005), which agrees with earlier research (Noble and Barry, 1982; Grela, 1996; Tveitnes, Singh and Ruud, 1996). However, the ancient diploid wheat, *Aegilops tauschii* L. and rye (*Secale cereale*) were found in our studies to be 42 percent and 35 percent higher ($p < 0.001$; $p = 0.03$), respectively, in grain Se concentration than other cereals in separate field trials, and in a hydroponic trial rye was 40 percent higher *(*$P < 0.001$) in foliar Se content than two wheat landraces. *Ae. tauschii* was also higher in Zn, Fe and Mn than other wheats in the trial. Other wild wheat relatives may also be efficient accumulators of Se and other minerals (Piergiovanni *et al.*, 1997).

Studies of genotypic variation of I in diverse plant species have yielded variable findings. A Japanese survey found no significant difference in leaf I concentration for plants grown on similar soils (Yuita *et al.*, 1982), while an earlier survey of different pasture species grown together in the field found a thirty-four-fold variation in leaf I concentration, with perennial ryegrass (*Lolium perenne* L.) the most efficient I accumulator (Johnson and Butler, 1957).

Evidence is scarce for significant genotypic variation in grain density of I in wheat (Shinonaga *et al.*, 2001), and no variation was detected in our South Australian trial, where varieties were grown at three locations, with three replications. I concentrations were low, typically less than 20 µg/kg (range 10–60 µg/kg DW) in whole grain (Lyons *et al.,* unpublished).

Maize

Little research has been conducted on genotypic variation in Se or I concentration in maize kernels. Our group has conducted a limited survey of diverse maize genotypes grown in the United States of America (Illinois) and Nigeria. No significant variation was detected for either micronutrient; however, the soils at both sites were low in available Se, resulting in kernel Se levels at the Nigerian site, for example, of just 5 µg/kg DW (range 3–10 µg/kg DW). Such low levels may have limited expression of possible varietal Se differences (Lyons *et al.*, unpublished).

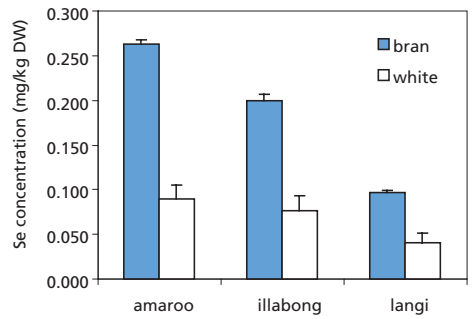
Rice

Rice appears to be a more promising cereal for genotypic variation in Se, with differences detected in other studies (Nan and

Han, 1993; Zhang *et al.*, 2004) as well as our own, which involved several varieties grown together in New South Wales, Australia. Three varieties differed in Se concentration in bran (means [SE] of 97 [12], 200 [17], 263 [14] µg/kg DW), while one was lower than the others in endosperm Se concentration (40 [2], 83 [5] µg/kg DW) ($P < 0.001$) (Lyons *et al.*, 2005b; Figure 17.3).

Genotypic variation in I concentration in rice bran was apparent in our Australian study, with all three cultivars different ($p = 0.02$). The mean I concentrations in the bran [SE] in µg/kg DW were 910 [50], 770 [10], and 500 [50]. There was no difference in I concentration in the endosperm for the three cultivars (Lyons *et al.*, unpublished).

FIGURE 17.3
Effect of genotype on Se concentration in rice bran and white (polished) rice. Bars represent means of three replications ± SE



Source: Lyons *et al.*, 2005b.

17.3 GENOTYPIC VARIATION IN STAPLE CROPS FOR DISTRIBUTION OF MICRONUTRIENTS WITHIN EDIBLE COMPONENTS

17.3.1 Fe & Zn

Wheat

Almost all studies to date have dealt with nutrient concentration in the whole grain, and there are few data available on the distribution of micronutrients in different grain fractions. Lyons *et al.* (2005c) studied distribution of grain Fe, Zn and other nutrients across the seed tissues of four wheat genotypes, and reported that the proportion of Zn in the grain (percent of total grain Zn content) was in the following order: endosperm (including aleurone layer) > embryo > bran (72–78, 11–27 and 2 percent, respectively). The proportion of Fe in the grain fractions followed the same order as Zn (79–91, 3–19 and 2–9 percent for endosperm, embryo and bran, respectively).

Rice

The highest proportion of Fe was located in pericarp, including aleurone layer (43 percent), followed by endosperm (42 percent) and embryo (10 percent) (Boyd *et al.*, 1972). The proportion of Zn was 60, 42 and 14 percent in pericarp (including aleurone layer), endosperm and embryo, respectively. It is interesting to see that when all proportions for Zn are added up, we get a value of over 100 percent, which may be due to contamination during dissection, processing or analytical errors associated with a very small sample size (Boyd *et al.*, 1972).

The distribution of nutrients in cereal grains is important for human nutrition. For obvious reasons, high concentration in the endosperm is desirable as endosperm makes up the majority of the grain. It is well known that a significant proportion of nutrients is lost in milling residue (Burk and Solomons, 1985). A further reduction in nutrient content occurs in the polishing process in rice (Graham *et al.*, 1999). Therefore, in breeding programmes, selection for higher Fe and Zn concentration in the endosperm would not only result in lower losses of Zn and Fe, but enhance bio-availability due to lower levels of phytate and fibre

components in endosperm than in bran (Lyons *et al.,* 2005c). Further research with a large number of genotypes is required to determine the extent of genotypic variation in distribution of Zn in the grain, and also to assess the potential for breeding for this trait.

17.3.2 Se & I

As noted above, genotypic variation was found by our group in rice bran and, to a lesser extent, endosperm for Se, but only in bran for I. Se concentration in rice bran was around three times that in endosperm, while I concentration in bran was around nine times that in endosperm (mean 730 vs 80 µg/kg DW) (Lyons *et al.,* 2005b). As most rice is eaten in the polished form, with the bran removed, selection for a higher proportion of grain Se and I stored in endosperm may be worthwhile, as noted for Zn and Fe above.

17.5 GENOTYPE × ENVIRONMENT INTERACTIONS AND THEIR EFFECTS ON MICRONUTRIENT TRAITS

G×E interaction is an important issue for plant breeders. A significant G×E interaction implies that rankings of genotypes differ with environment, indicating the need for testing at various sites or seasons. For example, a genotype may exhibit high-micronutrient-density traits in one environment, but not in others. A significant G×E interaction can be classified as either (i) non-crossover, where the ranking of genotypes remains consistent in different environments and it is the degree of accumulation that is affected; or (ii) crossover, where the rank of individual cultivars is affected by the environment (Baker and Kosmolak, 1977). G×E interactions and statistical methods for analysis and interpretation of G×E interactions have been reviewed elsewhere (Kang, 1990; Hill, Becker and Tigerstedt, 1998; Annicchiarico, Chapter 20 this volume). From a breeding point of view, it is important to understand the nature and extent of G×E interactions for designing breeding strategies and selection procedures (Eiseman, Cooper and Woodruff, 1990). Much effort has been directed to understanding G×E interactions in relation to yield, but little attention has been given to grain nutrient density. Graham *et al.* (1999) recognized that environment (soil type, fertilizer management and climate) can have a strong influence on nutrient density of grains; thus it is important to grow out the seed to be compared for at least one generation in the same environment to minimize the variation in nutrient density associated with previous growing conditions. Only then can valid comparisons of genetically controlled variation be made.

Fertilizer management can also influence micronutrient density in the grain. In studies with maize varieties grown at different nitrogen (N) and water levels, Feil *et al.* (2005) found that N fertilization reduced grain concentrations of Zn and Mn, which was attributed to higher grain yield as a result of N application. In contrast, continuous irrigation did not affect grain nutrient density. However, the rankings of varieties remained unchanged by water regime and N levels, pointing to stability of varietal differences in grain nutrient density over a range of N and water levels. At present, there is little information available on the effects of fertilization on grain micronutrient density in rice or wheat.

17.5.1 Fe & Zn

Wheat

From field trials conducted by our group in different locations and years

TABLE 17.3
Fe and Zn concentrations (mg/kg DW) in grains of wheat cultivars grown at different locations and years in Australia

Cultivar	Zn concentration						Fe concentration					
	Lameroo 1996	Bute 1997	Birchip 1998	Horsham 1998	Birchip 1999	Birchip 2000	Lameroo 1996	Bute 1997	Birchip 1998	Horsham 1999	Birchip 1999	Birchip 2000
Excalibur	22	18	21	17	25	18	37	32	44	35	40	37
Krichauff	20	19	25	18	24	18	36	34	46	38	39	38
Songlen	24	20	25	19	31	16	37	35	45	38	42	35
Trident	18	17	25	19	23	17	35	32	45	35	38	38
Yallaroi	20	17	22	16	24	14	32	32	45	36	40	37
Mean	21	18	24	18	26	17	35	33	45	36	40	37
Standard deviation	2	1	2	1	3	2	2	1	1	2	2	1

Source: Graham *et al.,* unpublished.

TABLE 17.4
Fe and Zn concentrations (mg/kg DW) in grains of wheat cultivars grown at two levels of Zn fertilization at Lameroo (1996) and Bute (1997) in South Australia

Cultivar	Zn concentration						Fe concentration					
	Lameroo			Bute			Lameroo			Bute		
	Nil	+Zn	Mean*	Nil	+Zn	*Mean	Nil	+Zn	*Mean	Nil	+Zn	*Mean
Barunga	14	22	18	15	20	17	38	36	37	33	31	32
Cascades	14	21	17	11	20	15	38	35	36	35	31	33
Excalibur	14	22	18	11	18	14	36	37	36	34	32	33
Frame	12	19	15	12	19	15	32	31	32	35	34	35
Halberd	13	23	18	12	17	14	35	33	34	36	35	36
Janz	12	18	15	11	16	13	29	29	29	29	27	28
Krichauff	12	20	16	12	19	15	38	36	37	37	34	35
RAC750	12	22	17	13	21	17	33	31	32	31	31	31
RAC809	11	19	15	10	16	13	36	35	35	34	30	32
RAC812	11	17	14	12	20	16	33	31	32	35	34	34
RAC820	15	22	18	10	15	13	33	33	33	34	32	33
RAC826	13	21	17	12	18	15	33	34	34	36	33	34
RAC832	13	21	17	14	24	19	30	31	31	38	35	37
RH911996	15	21	17	10	16	13	32	33	32	31	31	31
RH912025	14	20	17	11	18	14	36	31	34	33	35	34
Songlen	14	24	19	11	20	15	36	37	37	36	35	35
Tammin	14	20	17	10	17	14	37	35	36	34	31	32
Trident	12	18	15	11	17	14	35	35	35	35	32	33
WI334	11	17	14	11	16	14	33	33	33	35	33	34
WI94063	11	18	15	13	19	16	31	30	30	29	30	30
WI94091	10	14	12	12	18	15	29	27	28	32	29	31
Yallaroi	17	21	19	12	17	15	34	32	33	32	32	32
Yanac	11	17	14	11	16	13	31	30	31	35	31	33
Mean	13	20		12	18		34	33		34	32	
Range	10–17	14–24		10–15	18–24		29–38	27–37		29–38	27–35	

NOTES: Genotype × Zn fertilization interaction for grain Zn and Fe concentrations was non-significant, while genotype × location interaction was significant ($LSD_{0.05}$=3 for Zn and Fe). +Zn treatment received granular zinc (zinc oxysulphate, 32 percent Zn) at a rate of 7 kg/ha at seeding and a foliar spray (Zincsol, 16.7 percent Zn) at a rate of 2 L/ha at the early growth stage.
Source: Graham *et al.,* unpublished.

in Australia, Zn application resulted in an increase in grain Zn concentration in all varieties and sites. However, few genotypes in these trials were retained year after year, thus G×E interactions could not be analysed for all environments. However, when we analysed grain Zn and Fe data for the five genotypes tested in 6 environments (Table 17.3) or 23 genotypes tested in two environments (Table 17.4), responses of genotypes differed with environments (locations and years), indicating the presence of G×E interactions. When we subjected the data for grain Zn concentration (adequate Zn only) in Table 17.5 to Spearman's Rank Correlation Test (r_s), we found a non-significant correlation between rankings of genotypes in two different environments (r_s = 0.223), suggesting that rankings of genotypes differ with environment.

Most recently, significant G×E interactions were also reported for grain Zn and Fe concentrations, which would make direct selection for these traits difficult (Oury *et al.*, 2006). The ranges in grain Zn and Fe concentrations of adapted material in this study (15–35 and 20–60 mg/kg DW for Zn and Fe, respectively) were wider than those found in our field study (Table 17.5), which might be attributed to differences in genotypes and environments between the two studies. These limited studies suggest that there is a need for further field trials at different locations and years to determine or confirm the extent and nature of G×E interactions and their effects on grain Zn and Fe concentrations in wheat.

Rice

It was reported that high Fe and Zn traits were expressed in all rice environments (Graham *et al.*, 1999) and G×E interactions were sufficiently moderate (Gregorio *et al.*, 2000), suggesting that breeding for high Fe and Zn traits is a worthwhile effort. However, there was some evidence of G×E interaction in extreme environments. Although these limited studies are encouraging, there is clearly a need for further studies in this area.

17.5.2 Vitamin A

No G×E effect on lutein concentration was reported by Matus-Cadiz *et al.* (2003) when they investigated the effect of genotype, year and location in Australian and Canadian wheat varieties. However, further statistical analysis of the data revealed that 12 of the 79 cultivars showed significant crossover genotype-by-year interactions, indicating that in different years those cultivars reported changed in lutein concentrations that affected their rank.

17.5.3 Se

While genotypic differences may exist in modern wheat varieties, they are likely to be small in comparison with background soil variation. Soil Se is uneven in distribution and availability, with total Se concentrations ranging from less than 0.1 to more than 100 mg/kg DW (Berrow and Ure, 1989). Areas that are notably low in Se include parts of China, Siberia, central Africa, eastern Europe and New Zealand (Combs, 2001). In studies of grain Se concentration in wheat grown in South Australia, our group has found substantial microspatial (that is, metre-to-metre) variation in levels of available Se in soils. For example, at one trial site near Bordertown, south-east of Adelaide, we found a six-fold variation in grain Se concentration in four replicates of one wheat cultivar, grown together in the same field (Lyons *et al.*, 2004). Hence

the detection of what may be relatively small (for example, 10 percent) genotypic variations in Se uptake efficiency between wheat cultivars under these field conditions is virtually impossible. Background soil variation in available I has also been found to be substantial at the South Australian sites we have used, although less so than for Se. This large microspatial variation in soils makes it difficult, if not impossible, to accurately assess genotypic differences across environments for Se and I. This and narrow genotypic variation reported so far may be the reasons why to date there have been no studies reported on G×E interactions for Se and I.

17.6.SCREENING AND ANALYTICAL METHODS FOR MICRONUTRIENTS IN FOOD CROPS

Where should screening be carried out: field or greenhouse? The principles of both controlled environment and field screening are reviewed elsewhere (Graham, 1984), and therefore will not be dealt with in detail here.

17.6.1 Fe & Zn

The data presented in Table 17.4 suggest that screening for grain Zn concentration should be carried out in optimal growing conditions, as variation in grain Zn concentration under Zn deficient conditions is rather narrow. Unlike traits such as agronomic Zn efficiency, screening at the early growth stage does not appear to be suitable for detecting or identifying genotypes with the ability to load more Zn into the grain, due probably to the overriding importance of re-mobilization of Zn from leaves into grain occurring towards maturity. The evidence for this comes from a study in barley (Lonergan, 2001) in which two of the four chromosomal regions (also known as Quantitative Trait Loci, QTL) identified were found to co-segregate for grain Zn accumulation and vegetative Zn accumulation at anthesis, indicating little prospect for screening for grain Zn accumulation even as early as anthesis. Moreover, the only QTL detected for shoot Zn concentration or content (chromosome 4) did not co-segregate with Zn concentration or content at either anthesis or maturity, suggesting that screening for grain Zn accumulation at the early stage will not be reliable or relevant to grain Zn accumulation.

Inductively Coupled Plasma Optical Emission Spectrometry (ICPOES) is commonly used to determine mineral nutrient concentrations in plant tissues, and allows the determination of interactions among the various essential nutrients, effects that can be large and important. This method is fast and reliable, but can be costly for breeding programmes in both developed and developing countries, where tens of thousands of samples are handled each year. Are there alternative and cheaper methods to ICPOES? The researchers at the University of Adelaide (Australia) have developed a rapid, cheap and user-friendly assay for determination of Fe in the grain of rice or wheat (Choi, Graham and Stangoulis, 2007). This new cost-effective assay consists of two phases. In phase one, the assay is used to identify high grain-Fe lines from thousands of samples, while in phase two, the high-Fe lines identified in phase one are confirmed by ICPOES.

17.6.2 Vitamin A

Several standard procedures for extraction and identification of carotenoids from plant material have been used since the discovery and naming of carotene in the early 19th century. Separation of carotenoids initially involved a two-step chromatographic

method involving open-column and thin-layer chromatography (TLC). These two methods have been combined in high performance liquid chromatography (HPLC), which is now the preferred method for carotenoid analysis.

Spectrophotometric analysis, TLC and HPLC all require extensive extraction procedures using organic solvents that are both costly and toxic. While there is no doubt that these methods are necessary for elucidation of specific isomers and absolute quantitative analysis, this reduces the scope for identification of high-carotenoid parent lines. Given the participatory focus of this book, a fast and accurate method of identifying high carotenoid concentrations would vastly increase the number of lines that could be screened to identify suitable parents by persons with little organic chemistry background.

Spectrophotometric determination of wheat grain xanthophyll concentration following extraction of flour or meal with water-saturated butanol is well established (AACC, 1983). Similarly, reflectance spectrophotometric measurement of flour colour is commonly used (Oliver, Blakeney and Allen, 1992), as is the relationship between Commission Internationale l'Eclairage (CIE) b* and extractable yellow pigments (Mares and Campbell, 2001). Colour determined by CIE classifies colour in three dimensions: L*, brightness; a* red to green colour; and b* yellow to blue colour. CIE colour is influenced by inherent genotypic characteristics, environmental conditions and stresses during grain production, the milling procedure and by the size of flour particles and bran flakes, which is caused by differences in grain hardness and moisture content of the grain at milling. Variation in L* affects the measurement of b* and potentially could result in errors in estimating carotenoid content (Mares and Campbell, 2001).

Current methods for the identification of wheat genotypes high in specific carotenoids involve HPLC and are slow, costly and highly labour intensive. The chemical structure of carotenoids indicates that a correlation with colour is likely and it is therefore possible that divergent selection for colour in bread and pasta wheat has influenced the carotenoid content of these species. Determination of a correlation between a fast and accurate colour measurement, such as that obtained from the Minolta Chroma Meter, and carotenoid concentration determined by HPLC, could vastly increase the number of samples that could be screened in a given period.

In a recent report (Humphries *et al.*, 2004), whole-meal wheat, including both bread and durum varieties, and triticale samples were analysed for their carotenoid content by HPLC, and also for colour using reflectance spectrophotometry (CIE L*a*b*). A positive correlation between CIE b* (yellowness) and lutein concentration was shown in all wheat groups, but was strongest in the durums. There was little correlation between CIE L* (lightness) or CIE a* (redness) and lutein, α- or β-carotene. By contrast, the b* value correlated well with the concentration of α- and β-carotene, and therefore the vitamin A activity, though those wheat groups that did not have a strong correlation were those with the lowest CIE b* values. The durum wheat had the highest CIE b* value and the highest lutein concentration, but a relatively low concentration of β-carotene.

17.6.3 Se & I

Because of the high soil variation in available Se and I, screening for higher

Se and I traits in cereals needs to include hydroponic trials and pot trials using a standardized growth medium, backed up by field studies conducted on soils that are relatively uniform in Se and I. Selenate is the most mobile Se form and the dominant available Se form in well-aerated, neutral to alkaline soils (Cary and Allaway, 1969), while selenite is the major form taken up by rice in flooded paddy soils of lower pH (Wang and Gao, 2001). Thus the composition of hydroponic culture media needs to be tailored to the relevant field situation. Using solution culture containing selenite as the dominant available Se form, Zhang *et al.* (2004) have found genotypic variation in Se concentration in the leaves of rice seedlings, and the levels are well correlated with those in grain.

Genotypic variation in I uptake in rice may be explained by differences in the oxidising power of the roots, which can oxidise the iodide ion to form molecular I, which is then absorbed more readily. A significant correlation was found between the oxidising power of rice roots and the uptake of I (Yamada *et al.,* 2005), hence this may prove to be a suitable screening method.

Commonly used methods of Se analysis include hydride ICPOES, ICP mass spectrometry, and fluorimetry. Sample preparation for hydride ICPOES involves digestion with a nitric+perchloric acid mixture, followed by hydrochloric acid digestion, then treatment with sodium borohydride (Tracy and Moller, 1990). ICP mass spectrometry, in which plasma is used as the ionization source, is a highly sensitive method for Se and I analyses (Hieftje and Vickers, 1989). Another commonly used (and time-honoured) method for both Se and I analysis is the fluorimetric method. This is based on the reaction of 2,3-diaminonaphthalene (DAN) with Se (IV) to form a fluorescent Se-DAN complex, piazselenol (Koh and Benson, 1983). Samples for I analysis are typically prepared using tetramethyl ammonium hydroxide (TMAH) extraction.

17.7 INHERITANCE OF MICRONUTRIENT ACCUMULATION IN FOOD CROPS

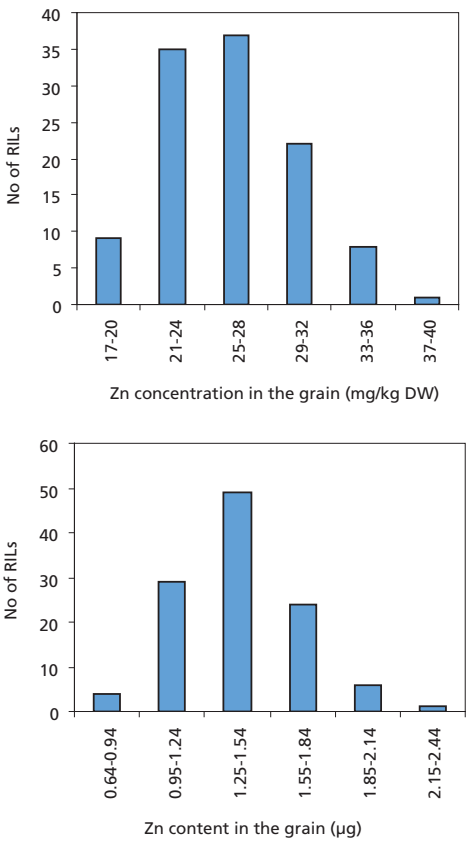
Apart from the existence of genetic variation, breeding for enhanced grain nutrient content also requires knowledge of genetic control mechanisms.

17.7.1 Fe & Zn

Wheat

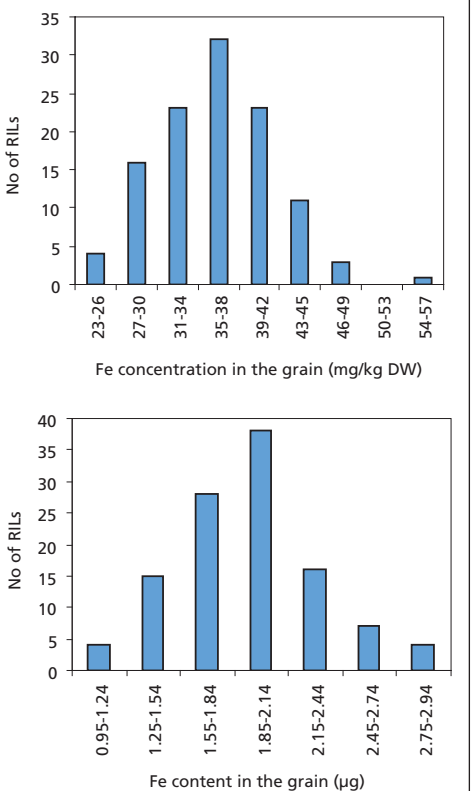
At present, there is little or no information available on genetics of Zn and Fe accumulation in wheat grain. The continuous variation in grain Zn and Fe concentration and content within Recombinant Inbred Lines (RIL) (n=113) derived from Opata × Synthetic cross (Figures 17.4 and 17.5) indicates that the two traits are quantitative and controlled by several genes (Genc *et al.,* unpublished). It is interesting to note that some RILs had higher Zn and Fe concentration and content than either of the parents, suggesting a transgressive segregation, which is probably due to these lines carrying favourable allele combinations from both parents. This multi-gene control hypothesis is supported by recent studies that identified several chromosomal regions associated with grain Zn concentration (Shi *et al*., 2007; Genc *et al*., 2009) and content (Shi *et al*., 2007). However, these field studies were conducted at a single location and QTLs identified were mapped to either different chromosomes or to different regions of the same chromosome. Therefore, there is a need to test mapping populations at multiple sites and years to validate the QTLs and also to determine the extent of GE interactions on grain Zn and Fe

FIGURE 17.4
Frequency distribution of grain Zn concentration and content in Recombinant Inbred Line population of Opata × Synthetic grown in standard potting mix in a glasshouse (Zn concentration, 24 and 20 mg/kg DW; Zn content, 1.29 and 1.08 µg/grain for Opata and Synthetic, respectively)



Source: Genc *et al.*, unpublished.

FIGURE 17.5
Frequency distribution of grain Fe concentration and content in Recombinant Inbred Line population of Opata × Synthetic grown in standard potting mix in a glasshouse (Fe concentration, 33 and 30 mg/kg DW; Fe content, 1.74 and 1.63 µg/grain for Opata and Synthetic, respectively)



Source: Genc *et al.*, unpublished.

traits. Identification and validation of QTLs associated with high grain Fe and Zn traits will accelerate breeding for these complex traits. Marker-assisted selection is discussed in Chapter 19 of this volume.

Rice

Genetic analysis of grain Fe concentration using four traditional varieties, three advanced lines and three IRRI-released varieties revealed the presence of a large genetic effect (additive and non-additive gene action) and small environmental effects (Gregorio *et al.*, 2000). Narrow-sense and broad-sense heritabilities were 44 percent and 88 percent, respectively. This study suggested that selection for a high grain-Fe trait should be delayed as late

as F_5 generation where dominance effect is not evident. This study also identified three chromosomal regions associated with a high grain-Fe trait (chromosomes 7, 8 and 9), providing evidence for multi-gene control for this trait, in which case, selection as late as F_5 may mean that lines bearing the fullest expression of high Zn concentration may be few in number and so lost in earlier generations unless populations are large.

17.7.2 Vitamin A

Maize

It is important to be aware of reciprocal differences in their contribution to kernel content of carotenoids, as this will affect the inheritance of the traits. Several studies have shown that the pollen parent affects carotenoid concentrations of the F_1 seed of reciprocal crosses (Mangelsdorf and Fraps, 1931; Johnson and Miller, 1938; Randolf and Hand, 1940; Grogan *et al.*, 1963)

However, a study by Egesel (2001) found that the female parent had the greatest influence on carotenoid concentrations in open pollinated kernels. Another study reported broad sense heritability of 33 percent for β-carotene and 47 percent for β-cryptoxanthin (another carotenoid with provitamin A activity) (Wong, 1999).

To produce colour in the maize kernel, numerous genes are necessary for structural and regulatory mechanisms. For carotenoid production, three genes have been reported to be relevant to carotenoid concentration. The *Y1* (yellow 1) gene on chromosome 6 encodes for phytoene synthase (Buckner *et al.*, 1996), an essential enzyme in the carotenoid pathway; the *VP9* (viviparous 9) gene on chromosome 7 is associated with ζ-carotene desaturase; and the *VP5* gene encodes for phytoene desaturase (Wong *et al.*, 2004). Carotenoids are produced in the starchy endosperm, and because of this, the yellow or white colours are only seen when the aleurone layer is colourless. The endosperm is triploid in nature, two from the maternal parent and one from the paternal parent. Thus the maternal parent can give a good indication of the expected carotenoid concentration (Egesel *et al.*, 2003). This results in a heterozygote containing two dominant or two recessive alleles, resulting in phenotypic differences (Table 17.5). For example, in the monohybrid cross for *Y1*, the endosperm can be one of four genotypes, which produce variation in colour (Symcox, Shadley and Weber, 1987). The genotypic differences correlate to the colour of the endosperm, and to the carotenoid concentration.

TABLE 17.5
Genotype and phenotype of maize heterozygote, and contributions from paternal and maternal parents

Endosperm genotype	Maternal contribution	Paternal contribution	Phenotype
Y1Y1Y1	Y1Y1	Y1	Yellow
Y1Y1*y*1	Y1Y1	*y*1	Light yellow
*y*1*y*1Y1	*y*1*y*1	Y1	Pale yellow
*y*1*y*1*y*1	*y*1*y*1	*y*1	White

Cassava

It was initially reported that the inheritance of root colour was simple and that a single dominant gene was responsible for yellow colour (Hershey and Ocampo, 1989). However, since that report, inheritance for carotenoid concentration has been found to be under the control of two genes, one responsible for transport to the non-photosynthetic roots, the other for accumulation within these storage organs (Chavez *et al.*, 2000). These two genes do not function independently of each other, rather each affects the expression of the other, though the mechanisms behind this are as yet unreported. The two major genes

are also combined with other genes with smaller effects that affect accumulation.

Wheat

A study into the genetic origin of an increase in carotenoid pigments in the cross between a wild barley (*Hordeum chilense*) and durum wheat located this trait to the α-arm of chromosome 7H^{ch} (Alvarez, Martin and Martin, 1998). Screening of 35 lines with various Tritordeum lines revealed that although the presence of the H^{ch} genome is responsible for increased carotenoid concentrations in these lines it is difficult to predict the effect of the interaction between the barley and wheat genetics. *H. chilense* is therefore a useful but not entirely reliable source of increased carotenoid concentrations for *T. durum* (Alvarez, Martin and Martin, 1999).

17.8 PHYSIOLOGICAL AND MOLECULAR MECHANISMS OF MICRONUTRIENT UPTAKE, TRANSLOCATION, RE-MOBILIZATION AND ACCUMULATION

17.8.1 Fe & Zn

For a breeding programme to be successful, it is important to understand the processes leading to accumulation of nutrients in the grain. Obviously an increase in accumulation of Fe and Zn in the grain of any plant species will require higher uptake, translocation or re-mobilization from source (leaves) to sink (grain). The role of these processes in relation to accumulation of Fe, Zn and other micronutrients has been reviewed recently (Grusak, Pearson and Marentes, 1999); thus it will not be discussed in detail here. It is interesting to note that Fe has been the most studied micronutrient in rice, while Zn has been researched to a larger extent in wheat. There is some suggestion that increasing the levels of Fe-chelating agents (phytosiderophores, nicotianamine, organic acids), reducing agents (ferric reductase), enzymes and transport proteins in the root cells could enhance Fe uptake and transport (Grusak, Pearson and Marentes, 1999). However, higher uptake and transport does not necessarily imply higher accumulation in the grain (phloem loading). For example, a pea mutant of cultivar Sparkle (*brz*) accumulated 36-fold higher Fe in the leaves compared to Sparkle, but did not have higher Fe in the seeds (Grusak, 1994). The author concluded that the rate limitation to phloem Fe loading was due to an unidentified ligand species that would complex with Fe prior to phloem loading rather than the availability of Fe as substrate. This study was followed by the study of Marentes and Grusak (1998), who demonstrated that a second mutant of cultivar Sparkle (*dgl*) had 2.5-fold higher Fe concentration in the embryo compared to Sparkle (163 and 65 mg/kg DW, respectively). This mutant also had higher Fe concentration in the seed coat. The authors used radiotracer ^{59}Fe to determine the movement of Fe in the seed coat, and found that Fe was symplastically phloem loaded. They further suggested that Fe resided within the non-vascular seed coat cells, and that the cells at the inner surface of the seed coat may facilitate the release of Fe to the embryo apoplast. The form of Fe in the seed coat or embryo is still not known at present.

A recent study in rice suggests that nicotianamine (NA) and nicotianamine synthase (NAS) genes (OsNAS1, OsNAS2 and OsNAS3) are also involved in long-distance transport of Fe (Inoue *et al.*, 2003), apart from their roles in the release of low-molecular weight compounds, phytosiderophores, from the roots of graminaceous plants. This release of phytosiderophores solubilizes rhizospheric

Fe(III) thus increasing plant uptake of Fe, for example in rice (Takagi, 1976; Takahashi *et al.,* 2001). Most recently, a rice metal-NA transporter, OsYLS2, has been linked to phloem transport and translocation to the grain of Fe (Koike *et al.,* 2004). Increasing the expression of transporters such as OsYSL2 and OsNASs in rice and other species can enhance Fe, and to some extent Zn, accumulation in the grains. However, further studies are required to determine the extent of genotypic variation in NA concentrations and its relative contribution to Fe and Zn accumulation in several varieties with low and high Fe and Zn in the grain.

In contrast to Fe, there is little information available on Zn translocation in the plant and transport to the grain. An earlier study (Longnecker and Robson, 1993) suggested that organic complexes with citrate and malate may be important in re-mobilization of Zn in the phloem. A recent study indicates that this may not be the case, as no relationship could be found between the presence of complexes or ligands and loading of Zn into the wheat grain (Pearson *et al.,* 1996). However, this result does not rule out the possibility of other endogenous chelates that may play a role in the long-distance transport of micronutrients (Welch, 1995). At the same time, transport to the grain is thought to occur predominantly via the phloem (Pearson *et al.,* 1995), and is well regulated (Herren and Feller, 1997). This regulation of Zn transport has been reported by Pearson, Rengel and Graham (1999), who suggested that low-Zn grain was not a strong sink for Zn, while in the case of high-Zn grain, there may be a barrier preventing excessive accumulation in the grain. It has also been suggested that phytosiderophores and nicotianamine may facilitate Zn uptake and transport in the plant (Scholz, Seifert and Grun, 1987; Treeby, Marschner and Romheld, 1989; Zhang, 1991; Cakmak *et al.,* 1996), but this needs to be established in future studies.

The positive correlations between Fe and Zn concentrations in cereal grains provide evidence that both nutrients may be taken up and translocated to the grain through the same process. However, as the correlation coefficient is never 1, there must be other mechanisms specific to Fe or Zn uptake or translocation to the grain, which warrants further investigation.

Having briefly discussed uptake and translocation of Fe and Zn to the grain, two questions arise: "How much of the Fe and Zn in the plant ends up in the grain?" and "Where are these nutrients deposited in the grain?" Contrary to earlier suggestions of poor re-mobilization of Fe from vegetative tissues to the grain (4 percent in rice, Marr *et al.,* 1995; 20 percent in wheat, Miller *et al.,* 1994), a recent growth-room study with wheat reported that 77 percent of total shoot Fe was re-mobilized to the grain at maturity (Garnett and Graham, 2005). The lower re-mobilization of Fe in the earlier studies involving field-grown plants were attributed to (i) precipitation of Fe in the apoplasm (inactive Fe) at high concentrations, which may result in non-re-mobilization, (ii) saturation of either the grain loading or phloem loading, and (iii) contamination of plant tissues by soil (references in Garnett and Graham, 2005). Miller *et al.* (1994) reported that, in wheat, 70 percent of Zn in the leaves was re-mobilized to the grains, while only 42 percent of shoot Zn re-mobilized to the grain in the study of Garnett and Graham (2005). The differences in the amounts of Zn re-mobilized in these two studies may be due to differences in genotypes and experimental conditions. It has been suggested that,

in wheat, relatively large amounts of Zn are transported into crease or inner pericarp tissues via the crease phloem, and translocation to the embryo and endosperm continues throughout grain development (Pearson *et al*., 1998). As Zn status of the grain improves, more Zn is distributed to the inner pericarp and less Zn to the endosperm, outer pericarp and embryo (Pearson, Rengel and Graham, 1999).

17.8.2 Se & I

In most plants, uptake, transport and assimilation of selenate is the same as for sulphate, and leads to synthesis of selenocysteine and selenomethionine; selenocysteine is then incorporated into proteins (Lauchli, 1993). Hence, the transfer of sulphate/selenate transporter genes from a Se accumulator like *Astragalus bisulcatus* may be useful for phytoremediation of high-Se areas (Goodson *et al.*, 2003). However, this strategy may not assist with Se uptake on soils with low Se availability, where most Se is present as selenite, selenide and elemental Se forms (Cary and Allaway, 1969). Selenite absorbed by the roots undergoes a series of reduction reactions, including conversion to selenide, and finally a reaction with O-acetylserine to form selenocysteine (Tsang and Schiff, 1978). Because of shared transporters, sulphate in growth media inhibits uptake of selenate (Ferrari and Renosto, 1972), and sulphite may inhibit uptake of selenite, but further studies are required to confirm this.

Iodine species of lower oxidative state and molecular weight (iodide, -1 and 116, respectively) are absorbed more readily than the heavier, higher valency forms (iodate, +5 and 214, respectively) (Umaly and Poel, 1971). I is transported mostly in the xylem, hence little is re-translocated from the leaves into the grain, where most is stored in the bran layers and lost during milling or polishing (Muramatsu *et al.*, 1989). To date, little has been reported on the physiology of I in the plant system and further studies are needed, especially on the forms in which I is transported and stored.

17.9 CONCLUSIONS

17.9.1 Fe & Zn

There is substantial evidence for genotypic variation to justify breeding efforts towards developing high grain-Fe and -Zn varieties. However, our knowledge of genetics, physiological mechanisms responsible for high grain Fe and Zn trait and G×E interactions is very limited, and now it is time to focus on these areas. One important point we should mention is that these proposed studies should be supplemented by bio-availability studies in animals and humans. There have been some concerns with respect to poor bio-availability of these nutrients due to naturally occurring high phytate concentrations in the grains. However, a study in rats reported that bio-availability of Fe and Zn remained constant in low- and high-density genotypes of cereals and beans (Welch *et al.,* 2000). So it is a reasonable argument that there will be an increase in absorption of these nutrients as their concentrations increase in the grain, despite their low bio-availability compared to animal food sources, as observed in the Philippine rice study (Haas *et al.*, 2005), though in that study, the varieties differed simultaneously in Fe and Zn, giving rise to potential interactions in the gut. We believe that breeding for these traits is a worthwhile approach given the impact the small increment in absorption of these nutrients will have on the lives of billions of people who are reliant on staple food crops such as rice and wheat for their dietary requirements of Fe and Zn. Finally and importantly, if

breeding for high grain-Fe and -Zn traits is to be successful and the varieties adopted by farmers, the high grain-Fe and -Zn traits must be linked to high yield. This has been achieved in rice (Gregorio, 2002), and results from wheat trials are also encouraging (R.M. Trethowan, pers. comm.).

17.9.2 Vitamin A

Despite extensive selection against pigmentation in several staple foods, genetic variation for carotenoid concentration can still be revealed by screening varieties available from germplasm banks, as illustrated in this chapter. Even within those staple crops that have substantial concentrations of carotenoids, the value of screening ancient varieties for sources of higher accumulation is obvious. The time consuming and expensive nature of carotenoid analysis still remains a significant restriction, though recent progress in the development of fast screening methods for wheat will expedite mass screenings for this staple crop. Although much work has been done in elucidating sources of increased carotenoid concentrations in staple foods there is still much to do before we obtain concentrations that can alleviate vitamin A deficiencies. In addition, it is not merely enough to develop lines with high carotenoid concentrations; they must be adapted to local conditions, and also be culturally acceptable and the carotenoids bio-available.

17.9.3 Se & I

The limited investigations carried out to date suggest that rice may be the most promising of the major cereals for breeding to improve grain Se and I density, although further screening of all the major cereals may reveal more germplasm that can enhance these traits. For rice, in particular, further pot trials and field trials conducted at sites with different soil types and including a wide range of germplasm grown together are needed to confirm whether sufficient genetic variability exists to enable selection for uptake and grain loading efficiency of Se and I. Previous studies suggest that Se and I delivered through bio-fortified cereals are highly bio-available (Jiang, Cao and Jiang, 1997; Lyons *et al*., 2003).

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CHAPTER 18

Breeding for quantitative variables Part 5: Breeding for yield potential

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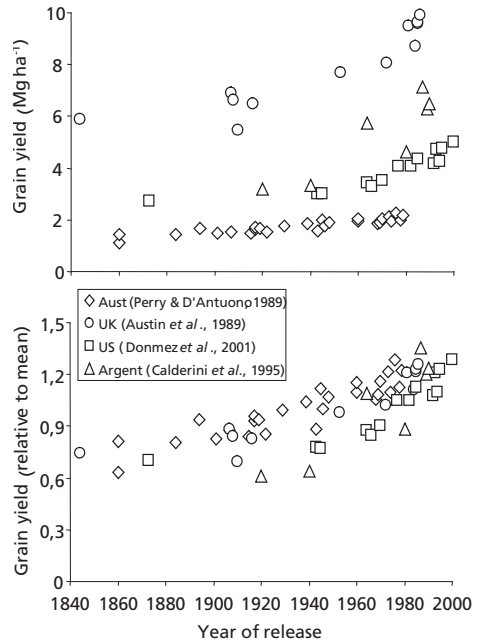
18.1 YIELD POTENTIAL

The combination of the continuing increase in the world's population and the lack of expansion, or even reduction, of arable lands (so as to maintain agricultural sustainability; Cassman *et al*., 2003), has lead to a situation where the relative importance of breeding to further raise yield potential and adaptiveness will be even greater than in the past (Slafer, Araus and Richards, 1999; Araus *et al*., 2002). This is no minor expectation: plant breeding in general, and cereal breeding in particular, have been remarkably successful during the second half of the twentieth century. They have substantially contributed to an increase in production at a rate faster than population growth, despite the Earth's population having increased faster than ever in the history of mankind (more than doubling in half a century). Despite increased demands for irrigation and chemical fertilizers, the technological progress made in cereal cultivation has actually led to a decline in the cost of cereal production per unit of output. Without this growth in productivity, people in many developing countries would have been forced to further extend cultivation onto marginal lands, thus aggravating the problem of how to sustain the natural resource base (IRRI, 1996). In the case of wheat, yield has been genetically improved virtually everywhere (Calderini, Reynolds and Slafer, 1999). The magnitude of the improvement has depended upon the environmental conditions of the region (Figure 18.1, top). However, when wheat yields are expressed as a percentage of the mean yield of the trial in which they were assessed, the data seem to converge on a single trend (Figure 18.1, bottom).

Nevertheless, the scenario is slightly more complex. Lately, yields have not been increasing at the pace seen from the 1950s to the 1990s (e.g. Calderini and Slafer, 1998; Conway and Toenniessen, 1999). As the population continues rising, and with it demand for human consumption (Reynolds, Sayre and Rajaram, 1999), food shortages will be unavoidable if food production increases do not return to previous rates, at least so as to match population growth (Khush, 1999). Therefore, increasing cereal yield is, even more so than in the past, an important challenge. If genetic yield potential is not increased in a sustainable manner, untouched natural ecosystems will go under the plough to meet greater demands, especially in the developing world.

Improvements in cereal productivity to meet the above requirement will not be easy without further technological

FIGURE 18.1
Yield of cultivars released in different years in Australia, United Kingdom, United States of America and Argentina in absolute (t/ha; top panel) and relative terms (bottom panel)



Source: Austin, Ford and Morgan, 1989

breakthroughs in shifting yield ceilings. The increase in cereal production has so far been obtained mostly from irrigated land, through the diffusion of improved varieties and agronomic practices suitable for specific ecosystems. Moreover, investment in irrigation has allowed the conversion of rainfed ecosystems when suitable. However, the rising costs of irrigation and the problems of management, cost recovery and the maintenance of existing systems constrain the further expansion of irrigation. Increasing siltation of reservoirs and canals, lowering of underground water levels, and the accumulation of salt in already irrigated soils are causing environmental concerns (IRRI, 1996). Furthermore, new growth sectors, such as industry and tourism, as well as increasing population and urbanization, are all competing for water resources. Moreover, sustainability concerns constrain the adoption of intensive agronomic practices (MacIlwain, 2004), bringing into question the desirability of the further expansion of irrigation (Araus, 2004).

Abiotic stresses frequently constrain the growth and productivity of major crop species such as cereals. They have been specifically covered in Chapter 16 and therefore we will only marginally refer to them here.

While genetic increases in yield potential are best expressed in optimal environments, they are also associated with better yields under drought (Trethowan, van Ginkel and Rajaram, 2002; Araus *et al*., 2002), nitrogen deficiency (Ortiz-Monasterio *et al*., 1997) and heat-stressed environments (Reynolds *et al*., 1998). However, this is disputed by several authors, as discussed in Chapters 3, 13, 14 and 16.

This chapter will discuss techniques for improving yield potential (and eventually adaptiveness to unfavourable environmental conditions) for small-grain cereals (such as wheat, barley and rice) and how a physiological understanding can contribute to reach such a goal. The study of crop physiology can assist cereal breeding in different ways: (i) improving the understanding of the factors that determine crop yield and adaptation through the pedagogical (for syncretic) concept of ideotype and, as a consequence, improving crop simulation models; (ii) defining particular 'secondary' traits to select for (analytical breeding) when choosing parents for crossing or screening in segregating populations; (iii) indicating the kind of genetically modified organisms (GMOs) that are worth developing and how to test them; and (iv) phenotyping associated with marker-assisted selection (MAS).

Special emphasis will be devoted to those aspects that may be particularly useful to the National Agricultural Research Systems (NARS) of developing countries, where research budgets are limited and prioritization is necessary. As such, we will focus on alternatives for evaluating secondary traits in an economical way, and prospects for the new array of molecular techniques available.

18.2 ANALYTICAL BREEDING

Genetic improvement may be achieved through selection either:

- directly, for a primary trait (such as grain yield) in a target environment (Ceccarelli and Grando, 1996). This has been referred to as empirical or pragmatic breeding; or
- indirectly, for a secondary trait, that must be putatively related to a higher yield potential or to an improved behaviour of the crop when it is grown in a stressful environment. This is known as analytical or physiological breeding.

Traditionally, breeders have achieved yield increases by intercrossing elite lines and selecting the highest- and most stable-

yielding offspring that express disease resistance and appropriate end-use quality. Thus, during the past 50 years, most of the progress in major cereals came from yield increases made possible through the gradual replacement of traditional tall cultivars by dwarf, and fertilizer-responsive varieties with superior harvest indices. These varieties were deployed as part of a package that included irrigation, fertilizers, pesticides and mechanization in a development strategy termed the Green Revolution, a term coined in March 1968 by William S. Gaud, the Director of the United States Agency for International Development (USAID).

The genetic and physiological bases governing yield are still quite poorly understood (Reynolds, Sayre and Rajaram, 1999), as yield is a quantitative trait under multigenic control, characterized by low heritability and a high genotype-by-environment (G×E) interaction (Jackson *et al.*, 1996). For these reasons, new and more strategic approaches must be explored if wheat yields are to keep pace with demand. Moreover, as empirical breeding seems to be reaching a plateau, different approaches, complementing empirical with analytical selection methodologies, may be needed to further improve grain yields. In such a context, analytical breeding, drawing on a physiological understanding of G×E interactions, may be an option. The multi-site testing of elite lines is unavoidable, and so the contribution of physiology to interpret the nature of G×E interactions, one of the critical drawbacks that the breeders have to face, may be crucial for future yield gains.

18.2.1 Identifying physiological traits

One approach to identifying potential secondary traits relies on selecting genotypes released as a result of previous breeding programmes. These genotypes are cultivated simultaneously under controlled conditions, thereby eliminating the effects on yield of varying management practices (Slafer *et al.*, 1994) and allowing the comparison of any physiological bases underlying the differences in yield capacity. Most of the traits identified in retrospective analyses have been shown to be constitutive in nature; that is, to be expressed in the absence of stress.

Retrospective studies: physiological changes associated with genetic improvement in grain yield

Understanding the contributions made in the past by successful wheat breeding may provide clues to help identify alternatives for breeders to further increase yield. Knowing the changes in physiological traits associated with genetic gains in yield potential is essential to improve the understanding of yield-limiting factors and to inform future breeding strategies. These studies may afford some clues regarding the physiological changes underlying the genetic gains in yield achieved in the past.

As well as the regular publication of this type of study, where cultivars released at different eras have been compared for yield and morpho-physiological determinants, there have also been several reviews, synthesizing the main findings from such studies (e.g. Calderini, Reynolds and Slafer, 1999). In the following section, we review the main attributes responsible for genetic gains in wheat yield in the past, including more recently published studies. The attributes are divided into four categories: time to flowering, and plant height; biomass production and partitioning; main yield components; and, lastly, cross-category interactions.

Time to flowering, and plant height

The timing of flowering is one major trait related to the adaptation of cultivars to particular growing areas, thus determining crop performance under the prevalent field conditions (e.g. Perry and D'Antuono, 1989; Passioura, 1996, 2002; Richards, 1996a; Slafer and Araus, 1998). This is why time to flowering (phenological adjustment) is one of the first attributes optimized by breeding programmes (Slafer, 2003). Consistent changes in this trait are therefore only to be expected in regions in which lengthening or shortening the growing season may have represented advantages for adaptation compared with cultivars released earlier. A scenario frequently reported in the literature, where the manipulation of time to heading may have a strong impact on adaptation, is that of regions characterized by a Mediterranean climate, i.e. a dry hot summer and humid, temperate winter (Perry and D'Antuono, 1989; Loss and Siddique, 1994; Acevedo *et al*., 1999). In these environments, the crop's vegetative and early reproductive phases occur under reasonably good water availabilities. However, as the season progresses, drought becomes more intense and frequent water stresses occur during the late reproductive phases. After anthesis, grains fill under rather severe water and heat stresses. The analysis of long-term trends in time to flowering for cultivars released in different eras reveals that, in most cases, there seemed to be little or no change in regions with climates different from the Mediterranean, while reducing time to flowering has been a successful strategy when breeding for environments characterized by terminal stresses. This is because earliness is probably the most effective solution for increasing yield where drought during grain filling is a common event (Passioura, 1996; Slafer and Whitechurch, 2001). Aside from its adaptive advantage, the reduction in crop duration has permitted an increase in cropping intensity for cereals such as rice, and also allowed land to be used for growing non-cereal crops in cereal-based farming systems (IRRI, 1996).

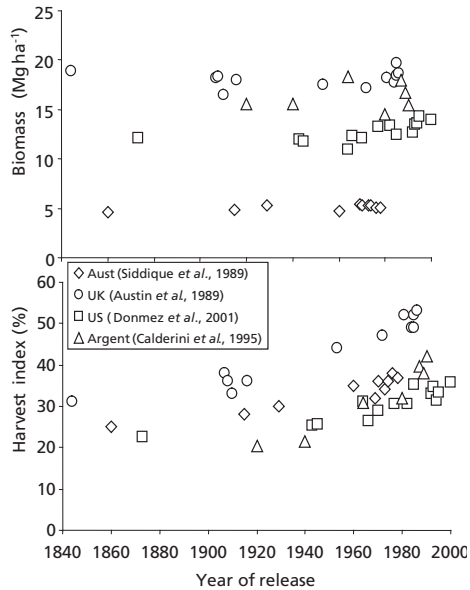
Breeders have always selected for reduced height when the initial elite material is taller than a threshold. For plant statures above this threshold (70–100 cm in wheat; Richards, 1992; Miralles and Slafer, 1995), there is no gain in biomass while there is a proportional reduction in harvest index. Below this threshold, the further gain in harvest index does not compensate for the loss in biomass, due to extremely poor radiation distribution within the canopy and consequent reductions in radiation-use efficiency (e.g. Miralles and Slafer, 1997). Thus, reducing height down to an optimum range increases yield potential (a similar biomass more efficiently partitioned to grains) and simultaneously reduces the risk of lodging causing yield penalties. Consequently, plant height has been reduced universally through wheat breeding during the 20th century (see a number of cases reviewed by Calderini, Reynolds and Slafer, 1999, and more recently reported studies: Donmez *et al.*, 2001; Brancourt-Hulmel *et al*., 2003; Jiang *et al*., 2003; Ramdani *et al*., 2003).

Reduced plant height has been one of the major causes of lack of adoption of modern varieties in a number of crops grown by farmers in developing countries as animal feed and where the way in which total biomass is portioned is irrelevant (Ceccarelli *et al*., 2000, Annicchiarico *et al.*, 2005).

Dry matter production and its partitioning

As yield has been greatly increased by genetic improvement during the past century (Figure 18.1) and grain yield is simply a

FIGURE 18.2
Biomass (t/ha; top panel) and harvest index (bottom panel) of cultivars released at different times in Argentina, Australia, United Kingdom and United States of America



Source: Austin, Ford and Morgan, 1989

fraction of the dry matter accumulated by the crop during the growing season, one might have expected a trend for increased biomass as newer higher-yielding cultivars have been released. However, in almost all cases in which the physiological bases of yield improvements have been analysed, final biomass was not associated with the year of release of the cultivars (Figure 18.2, top), with only a few exceptions to this general trend. Thus, for example, recent genetic gains in grain yield in the United Kingdom have resulted from a combination of improved growth rate in the pre-anthesis period, which has driven increases in the number of grains per unit area, and a larger source for grain filling through increases in stem soluble carbohydrate reserves (Shearman *et al.*, 2005).

While the lack of a consistent increase of biomass through breeding implies that neither leaf photosynthesis (Austin, 1989) nor radiation-use efficiency (Calderini, Dreccer and Slafer, 1997) are related to past yield increases, in a few cases positive relationships between maximum leaf photosynthesis and the year of release of cultivars have been reported (Fischer *et al.*, 1998; Jiang *et al.*, 2003). In the same way, Shearman *et al.* (2005) reported genetic increase across time for pre-anthesis radiation-use efficiency, which was correlated with wheat grain yield progress in the United Kingdom. However, except for that last study (Shearman *et al.*, 2005), these differences have not been associated with increases in biomass production. Looking in closer detail at post-anthesis, it seems, however, that modern cultivars may be characterized by higher radiation-use efficiency levels than their predecessors. This was the pattern observed in the only retrospective analysis of this characteristic that we are aware of (Calderini, Dreccer and Slafer, 1997). This increased radiation-use efficiency may just be a consequence of the increased demand of a larger sink in modern cultivars (see below) compared with their older counterparts. These kinds of results are in line with evidence from near-isogenic lines for semi-dwarfism (Miralles and Slafer, 1997) and with the fact that the number of grains growing after anthesis positively influences photosynthetic efficiency (Richards, 1996b). Also, it was recently reported that increased post-anthesis biomass may be achieved by increased sink strength through positive feedback to photosynthesis (Reynolds *et al.*, 2004). A higher stomatal conductance seems to be responsible, at least in part, for the higher photosynthetic rates (Fischer *et al.*, 1998; Araus *et al.*, 2002).

Therefore, the genetic gains in grain yield in most countries were virtually entirely due to modifications in harvest index (Figure 18.2, bottom), probably related to reductions in plant height (see above). In the few studies in which biomass partitioning was analysed before maturity in cultivars released in different eras, it seemed clear that the partitioning effects observed at maturity, as differences in harvest index, were already established by anthesis as variations in the spike-to-stem ratio (Siddique, Kirby and Perry, 1989; Slafer and Andrade 1993).

A critical issue in this respect is that harvest index in most modern cultivars seems to be close to its biological maximum (ca. 60 percent; Austin, 1980). Consequently, even though breeding in the past has been very successful in increasing grain yield through reducing height and increasing harvest index, it appears imperative to find alternatives for improving biomass—while maintaining harvest index—if further genetic gains in yield are to be expected.

Main yield components

The two main yield components are the number of grains per unit area and the averaged individual grain weight. Most experiments analysing genetic improvement effects on yield have considered these components. The vast majority of the reported studies found that, while selecting for higher-yielding cultivars, wheat breeders have consistently increased the number of grains per unit land area and produced either no trend or even a trend to slightly reduced individual grain weight (Calderini, Reynolds and Slafer, 1999; Donmez *et al*., 2001; García del Moral *et al*., 2002; Brancourt-Hulmel *et al*., 2003), though earlier studies have found a slight increase in this component during the 20th century (Cox *et al.,* 1988) or part of it (Calderini, Dreccer and Slafer, 1995).

The increased number of grains per unit and per area of the modern cultivars compared with their predecessors seems to be the consequence of a higher survival of floret primordia, as the number of potential florets per spike is apparently quite similar (e.g. Slafer and Andrade, 1993; Miralles *et al*., 2002). Thus, the higher survival of floret primordial—a process taking place during the last half of the stem elongation period (Kirby, 1988)—appears to be the most important factor leading to higher yield potential in modern cultivars (Slafer *et al*., 1994). This is in agreement with the finding that semi-dwarfing genes, which contribute significantly to increased yields in many breeding programmes throughout the world, increase the number of grains per unit land area by increasing the survival of floret primordia (Miralles *et al*., 1998). Actually, the giberellic acid-insensitive dwarfing genes *Rht-B1b* and *Rht-D1b*, the two most important commercially, have been reported to reduce plant height by around 18 percent, simultaneously exerting large pleiotropic effects improving spike fertility (Flintham *et al*., 1997).

Associations between attributes changed while selecting for higher yield

The genetic improvement of wheat yield may be understood more mechanistically by inspecting and interpreting the relationships between the main attributes of growth and partitioning of the yield components described above. Understanding the mechanistic bases by which breeding has successfully increased yield may shed light on possible future alternatives.

Genetic gains in grain yield were almost unequivocally due to gains in the number of grains per unit land area, with no gains and even slight losses in the average weight of the grains, probably as a consequence

of the lower size of the cellules induced by insensitivity to giberellic acid. This has given rise to a frequently found negative relationship between grain number per unit land area and the average weight of those grains (Slafer, Calderini and Miralles, 1996). In fact this negative relationship is also frequent when yield is increased by management practices. Although the most common interpretation in the literature has been an increased competition among grains as the number of grains per unit are is increased; we argue that the negative relationship is not competitive in nature (Slafer, 2003). This is because, even though wheat breeding has been reducing the degree of post-anthesis sink limitation to yield (e.g. Kruk, Calderini and Slafer, 1997), the photosynthetic capacity during grain filling together with the pre-anthesis assimilate reserves seem to be in excess of the demands of the growing wheat grains during post-anthesis (e.g. Richards, 1996b; Slafer, Calderini and Miralles, 1996; Borras, Slafer and Otegui, 2004; Reynolds *et al*., 2004). The main conclusion from this overall analysis is that, with only a few exceptions, wheat yield is limited by sink during grain filling and that further increases in yield depend upon increases in sink-strength after anthesis (to either further increase grain number per unit land area or to increase potential size of the individual grains).

The number of grains per unit land area is determined by various factors, including plants per unit land area, spikes per plant, spikelets per spike and grains per spikelet. These factors are sensitive to growing conditions throughout the entire period from sowing to anthesis (Slafer and Rawson, 1994). However, the number of grains per unit area seems to be far more sensitive to changes in growth partitioning over a rather short window of time immediately before anthesis (coinciding with stem elongation) than to any changes in growth occurring before this time (e.g. Kirby, 1988; Savin and Slafer, 1991; Fischer, 1993; Demotes-Mainard and Jeuffroy, 2001). In all these cases, there was a strong relationship between grain number per unit area at maturity and spike dry matter per unit area around anthesis (Slafer, 2003). Breeding seems to have increased grain number per unit area precisely through the same mechanism: crop growth has not clearly and systematically been affected by breeding for higher yield, but there has been a consistent trend to reduce plant height (a trait determined during the stem elongation phase), leading to increased partitioning towards the growing spikes resulting in an increased spike dry matter associated with more grains per unit land area (e.g. Siddique, Kirby and Perry, 1989; Slafer and Andrade, 1993). Studies demonstrating a consistent increase in yield caused by *Rht* genes in a wide variety of conditions also point to the importance of this mechanism (e.g. Brooking and Kirby, 1981; Fischer and Stockman, 1986; Miralles *et al*., 1998).

In this context, one can understand the positive relationship between the number of grains per unit area and harvest index that can be found almost without exception when comparing modern and old wheat cultivars. The number of grains is simply a reflection of changes in partitioning operating before anthesis, resulting in lower vegetative biomass.

18.2.2 Yield potential versus stress adaptation: G×E interaction

The limitations of empirical breeding are more evident when selecting for stress (e.g. drought) adaptation due to the existence of important G×E interactions, and higher within-site variability that also diminishes

heritability (h^2) (Richards, 1996a; Araus *et al*., 2002) even though this can not be generalized (Al Yassin *et al*., 2005; Comadran *et al*., 2008). Thus, although selecting for yield *per se* in the targeted environment may be sensible if the stress is uniformly severe (e.g. Ceccarelli and Grando, 1996), this is not the case in many realistic situations. For example, cultivars tested in a particular set of stressful conditions may not behave well in another set (Cooper *et al*., 1997). Moreover, a crossover effect in the yield of genotypes of high and low yield potential when regressed against the environmental index over a wide range of conditions is not often found unless the severe conditions are too extreme. This may indicate that in general, genotypes selected under high yielding environments will perform better than those with lower yield potential when grown in rather wide range of yielding environments (Calderini and Slafer, 1999; Slafer and Araus, 2007).

Constitutive whole-plant traits have a major role in affecting plant water use and plant dehydration avoidance under stress. These largely determine some of the negative relations between yield potential and the ability to sustain yield under severe water shortage (Blum, 2005). Under most dryland situations where crops depend on unpredictable seasonal rainfall, the maximization of soil moisture use is a crucial component of drought resistance (avoidance), which is generally expressed in lower water-use efficiency (WUE) (Blum, 2005) and may explain the positive correlations frequently found under Mediterranean conditions between carbon isotope discrimination ($\Delta^{13}C$, see below) and grain yield (Araus *et al.* 1998a, 2002, 2003c). However, selection for yield under drought stress resulted in a dehydration-avoidant phenotype that is rarely compatible with a high yield potential phenotype. If selection can address factors of stress adaptation in addition to yield under stress, perhaps higher yield potential and drought resistance can be recombined (Blum, 2005).

As mentioned earlier, this is one of the most controversial topics in plant breeding and the views presented in this paragraph represent one of the philosophies concerning G×E interaction. Different views and interpretations are discussed elsewhere in this volume (for example, Chapters 4, 14, 16 and 20).

Physiological avenues for increasing yield potential

As stressed above, retrospective studies with wheat indicate that improvement in yield has more often been associated with increased partitioning of biomass to the grain than it has with increased overall biomass (Austin *et al*., 1980; Waddington *et al*., 1986; Sayre, Rajaram and Fischer, 1997; Calderini, Reynolds and Slafer, 1999). Since harvest index is estimated to have an upper limit of just over 60 percent (Austin, 1980), and since this limit is already being approached (Shearman *et al*., 2005), it is becoming more important than ever to understand the physiological and genetic bases of radiation-use efficiency and biomass determination if yield is to go on increasing (Araus *et al*., 2003b).

Increases in biomass have started to be reported in spring wheat (Reynolds, Sayre and Rajaram, 1999) and winter bread wheat (Shearman *et al*., 2005). One study has revealed increases in biomass of about 10 percent in spring wheat specifically associated with the introduction of the long arm of chromosome 7D from a distant relative of wheat, *Lophopyrum elongatum,* into a number of wheat backgrounds (Reynolds *et al*., 2001). Detailed physiological investigation revealed that the basis of this increase in

biomass was associated with a small increase in assimilation rate during the spike growth stage, and a much larger increase in photosynthetic rate during grain filling, leading to an increased number of grains per spike (Reynolds, Pellegrineschi and Skovmand, 2005). Further experiments, in which grain number was increased artificially in elite lines with a brief light treatment during the rapid spike growth stage, showed that these lines posses a photosynthetic capacity in excess of that needed to fill the grains they would normally set (Reynolds, Pellegrineschi and Skovmand, 2005).

One way to exploit this excess photosynthetic capacity would be to increase grain number. CIMMYT is experimenting with a number of approaches, one of which being to exploit the large-spike trait. Large spikes themselves do not necessarily result in a higher yield should the trait not be in balance with other plant characteristics. For example, genotypes with large spikes often have small and shrivelled grain. Large-spike genotypes frequently tiller less, presumably because they carry the tiller inhibitor gene (Richards, 1988), which results in what is known as yield compensation. The challenge is therefore to bring traits together in a balanced way such that increased grain number is matched by an adequate vascular system with the ability to fill all of the additional grains, and a good tillering capacity is combined with large spikes.

Another trait being explored is the so called multi-ovary characteristic, which causes a single floret to set up to four kernels instead of just the usual one (Reynolds, Pellegrineschi and Skovmand, 2005). Currently the trait suffers from the problem of low kernel weight, but pre-breeding is underway with different spike architectures to try to better accommodate the large number of grains in terms of space and vascular connections. Traits that have shown association with improved yield in populations of random sister lines include above-ground biomass at flowering, spike mass at flowering, and duration of rapid spike growth phase (Reynolds, Pellegrineschi and Skovmand, 2005).

An alternative approach to further raise the number of grains per unit land area might be to lengthen the stem elongation phase (hypothesized by Slafer, Calderini and Miralles, 1996; Slafer *et al.,* 2001). The hypothesis would be that a longer stem elongation phase may result in more crop growth during this phase, higher spike dry matter at anthesis and subsequently more grains being filled. The hypothesis only makes for a viable solution if the length of the stem elongation phase can be manipulated and if the expectedly higher biomass accumulated during the phase is not counterbalanced by reduced partitioning to the spikes. These manipulations might involve genes responsible for sensitivity to photoperiod or for earliness *per se*, as the duration of the stem elongation phase seems to be governed by photoperiod response (e.g. Slafer and Rawson, 1997; Miralles and Richards, 2000; González, Slafer and Miralles, 2002) and to present genetic variation in its minimum duration, the intrinsic earliness (grown under long photoperiods after removing vernalization requirements; Slafer, 1996). Details of this hypothetical alternative can be found in Slafer *et al*. (2001). Briefly, there is clear genetic variation in the duration of stem elongation, even when holding constant the duration of the entire period to anthesis (Slafer and Rawson, 1994; Kernich, Halloran and Flood, 1997). At least part of this variation may be due to sensitivity to photoperiod. Such sensitivity varies between phenological phases (Slafer and Rawson,

1996; González, Slafer and Miralles, 2002). Exposing plots to photoperiod extensions during the stem elongation phase—natural day length was maintained before this phase—produced a change in the duration of the phase, associated with changes in the number of fertile florets and grains due to modifications in spike dry matter at anthesis (González, Slafer and Miralles, 2003a, b). This suggests that the mechanism by which photoperiod alters the final number of grains per unit area is the same as that determined by radiation interception during stem elongation (González, Slafer and Miralles, 2005). Therefore, isolating and subsequently manipulating (traditionally or through marker assisted selection) the genetic bases controlling sensitivity to photoperiod (as in this example, or genetic bases of differences in earliness *per se*) during stem elongation, might be an effective avenue for increasing yield.

18.3 THE PRACTICAL USE OF SECONDARY TRAITS

The putative secondary traits for a breeding programme assisted by analytical selection can be used:

- for the selection of parents to be included in the crossing block; or
- sas direct selection criteria for screening among a large number of genotypes (i.e. segregating populations) and when the amount of seed available is too small to carry out field trials with replications (i.e. the evaluation of double-haploid lines).

Whereas intensive work is continuously being carried out by physiologists to increase yield potential, few breeders routinely use the latest developed physiological criteria in their mainstream breeding programmes. One reason may be the difficulty in evaluating the response to the selection of secondary traits, this being an essential requirement for their incorporation into breeding programmes. The real value of a given trait may only be assessed by determining the genetic gain in segregating populations following selection. However, many traits are not available in well adapted genotypes, and their validation frequently requires the development of appropriate breeding material, which is costly and time consuming (Royo *et al.*, 2005). Moreover, the evaluation of some of the traits proposed by plant physiologists can be time-consuming, and sometimes even expensive, which is not practical for application to the thousands of entries that comprise the segregating generations of breeding programmes. In addition, selection in segregating populations requires screening at the plant level or between very small plots, thus hindering the use of traits that require large field plots to be assessed.

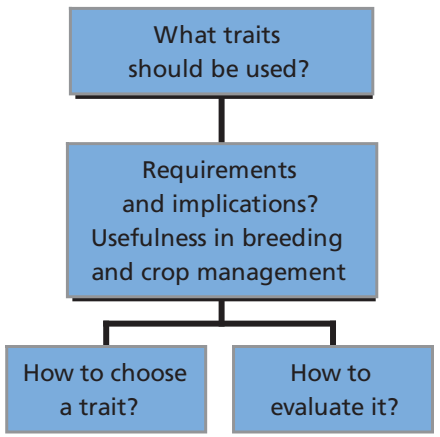
Nevertheless, some analytical or indirect selection criteria have been used for decades in breeding programmes. Plant height, days to heading or to maturity, photoperiod or vernalization responses, spike length, disease reaction, tillering capacity or grain weight are examples of traits usually evaluated in wheat and barley breeding programmes, both conventional and participatory (Ceccarelli *et al.*, 2000, 2003) so as to provide relevant information about the performance of genotypes.

Any trait to be chosen must fulfil a set of requirements related to relevance in terms of crop performance, as well as how it can be measured. These aspects are discussed below and summarized in several diagrams (Figures 18.3, 18.4 and 18.5).

18.3.1 How to choose a trait?

Two comprehensive manuals have been developed recently by CGIAR Centers for cereals such as wheat and rice. Both

FIGURE 18.3
The two main aspects when considering a candidate trait in a breeding programme



are free access. One (Reynolds, Ortiz-Monasterio and McNab, 2001) deals with how physiology may help in crop breeding, while the other (Fischer *et al.* 2003) refers to the overall context of a given breeding programme, where physiological traits could be used.

For a secondary trait to be useful in a breeding programme, it has to comply with various requirements (Araus *et al.*, 2002; Lafitte, Blum and Atlin, 2003) (Figures 18.3 and 18.4), including:

1. It should be genetically correlated with grain yield in the environmental conditions of the target environment, i.e. the relationship with yield has to be causal not casual.
2. It should be less affected by environment than is grain yield, i.e. it should have higher heritability than the yield itself, and so less G×E interaction.
3. Genetic variability for the trait must exist within the species.
4. In the case of traits addressed to breeding for stress-prone environments, the trait should not be associated with poor yields in unstressed environments. Unfortunately, this is the situation for many traits selected because they confer tolerance instead avoidance to a given stress (Araus *et al.*, 2002, 2003a).

FIGURE 18.4
Different requirements when choosing a candidate trait

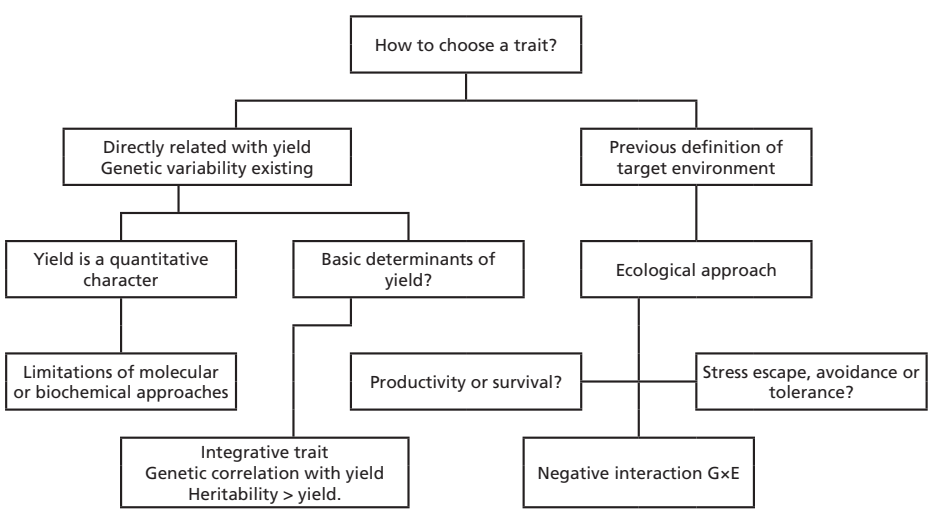
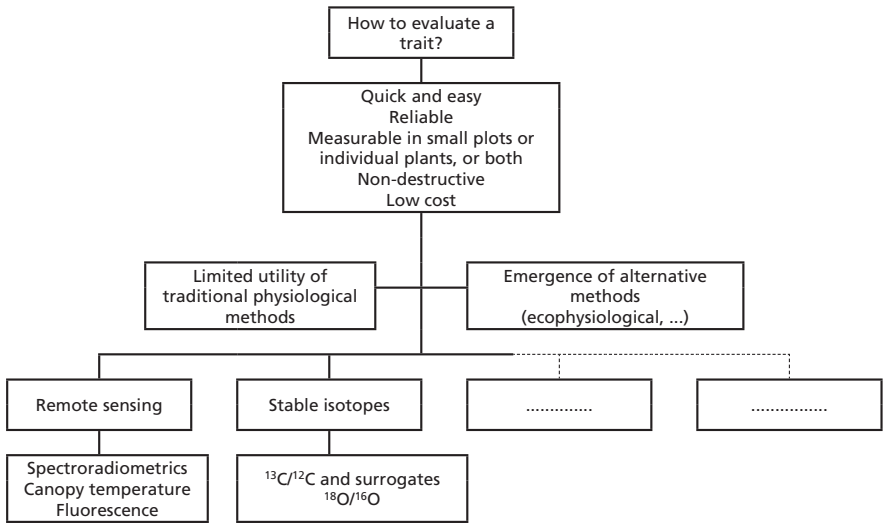


FIGURE 18.5
How a candidate trait should be measured



5. It should be possible to measure the trait rapidly and more economically than the yield itself, and in a reliable manner.
6. It can be assessed in individual plants or in very small plots.

We can predict whether the use of a secondary trait can enhance expected progress in selection by calculating its genetic correlation with yield (point 1) and heritability (point 2). Any trait that fulfils the first three requirements will provide the breeders with a useful prediction tool. While this may be enough for a breeding programme, for a direct confirmation of the value of a trait (validation), several approaches can be taken, including the development of lines expressing well the secondary trait and the assessment of their performance in the target environment, as well as the identification of the co-segregation of quantitative trait loci (QTLs) for the trait as well as for yield (Lafitte, Blum and Atlin, 2003). Once a given trait is chosen as a candidate, a practical way to incorporate it into a breeding programme is necessary (i.e. to fulfil point 5). This may be particularly pertinent when evaluating germplasm in segregating populations.

Secondary traits may be particularly suited to improving selection response for stress conditions, if they: (i) improve precision (in those case where the heritability of yield is reduced by stress); (ii) avoid any confounding effects of stress timing in yield (e.g. drought and flowering dates); (iii) focus the selection on a specific type of stress; and (iv) are cheaper, easier and faster to measure than grain yield.

Moreover, when choosing traits, it is necessary to keep in mind an eco-physiological perspective. For example, in the case of drought, most traits proposed by stress physiologists appear to be associated with stress tolerance (i.e. tolerate cell dehydration). If the target environment is under severe stress-prone conditions it may be helpful to select for this kind of trait. In most circumstances, however, the

main effect of drought is to reduce grain yield without killing the plant. In such cases, breeding for higher yield potential plus traits conferring stress avoidance (i.e. to avoid cell dehydration) may be in general a better choice (see examples in Araus *et al*., 2002, 2003a, b) but there is ample evidence that this is the exception rather than the rule (Chapter 16). At the same time, and indeed in most cases, trait evaluation should be carried out under field conditions, avoiding those experimental situations (growth chambers, greenhouses, pots) that are far from the agricultural growing environment.

18.3.2 Which traits to use in practice?

While many traits have been studied for their use in breeding for drought resistance, there is a general consensus among breeders that only a few of them can be recommended for use in practical breeding programmes at this time. For example, CIMMYT (Reynolds, Ortiz-Monasterio and McNab, 2001) and IRRI (Lafitte, Blum and Atlin, 2003) recommend the use of flowering and maturity dates, spike fertility, changes in green biomass (e.g. leaf death score) and canopy temperature. The manuals developed by both institutions include a comprehensive explanation of how to measure these traits. In practical terms, these traits seem valuable when breeding for higher yield potential and adaptation to moderate degrees of stress. Development of modern equipment and new analytical tools should facilitate future measurements of additional physiological traits in the field.

Phenology

This is the most widely used of secondary traits, due to its ease of measurement (see section 18.2.1. in this chapter). If the pattern of water deficit is predictable in a given region, selection for a flowering date that does not coincide with the period of water deficit (i.e. it exhibits an escape strategy) is a very effective way to improve drought adaptation (Araus *et al.*, 2002). The limitations to this approach are that very early varieties may suffer yield penalty in good seasons, while late-in-season freezing episodes may affect spike fertility.

Spike fertility

When stress occurs near flowering, the most sensitive growth stage, the main yield component affected is the percentage of fertile spikelets. This trait, important for any cereal, is critical for rice under water stress (Lafitte, Blum and Atlin, 2003).

Plant growth and senescence

Stress may accelerate the senescence of leaves. To measure leaf desiccation, it is possible to make a visual integration of the symptoms, translated to a ranking. Also, to check for early senescence of leaves, particularly the flag leaves, portable chlorophyll meters are extensively used. At the same time, stresses such as drought strongly affect leaf expansion (Royo *et al.,* 2004) and thus plant growth (Villegas *et al.*, 2001) and further yield (Araus *et al.,* 2002). Therefore the total green biomass evaluated at a critical plant stage (i.e. anthesis) or its change over time is a potentially powerful traits. A feasible evaluation of total biomass is only possible in practice through indirect methods, such as using spectroradiometers to measure the spectra of light reflected by the canopy (Aparicio *et al.*, 2000; Araus, Casadesús and Bort, 2001; Royo *et al.,* 2003). In many cases, however, the wide range of different spectroradiometrical indices have not fulfilled expectations when evaluating field plots for yield and their adaptation

to environmental conditions. The scarce use of spectral reflectance measurements as tools for screening in breeding programmes may be attributed to several reasons: (i) a wide range of variability for the measured trait must exist within the set of genotypes in order to be detected by the apparatus (Royo *et al*., 2003); (ii) the devices commercially available nowadays only allow measurements at canopy level, i.e. on medium to large plots, while, as noted above, selection in early segregating generations is based on individual plants or spikes cultivated in small plots; and (iii) the misleading use of spectral reflectance indices. Aside from the 'classical' vegetation indices related to green biomass (i.e. Normalized Difference Vegetation Index – NDVI; Simple Ratio Vegetation Index – SR), other indices are strongly affected by differences in green biomass (Araus, Casadesús and Bort, 2001). Therefore, the information provided by indices such as water index (WI – a measure of plant tissue content) or photochemical reflectance index (PRI – a measure of photosynthetic efficiency) is confounded by differences in biomass. Indices other than vegetation index only allow one to track physiological changes (i.e. in photosynthetic efficiency, pigment content and so forth) when differences in biomass do not exist across accessions or when used to track changes over time as a response to stress (see Tambussi *et al.,* 2002 for PRI). As an alternative to the use of indices, models constructed using the complete VIS/NIR reflectance spectra have proven to be accurate in ranking durum wheat genotypes by their grain production, although they did not provide a proper quantification of yield (Ferrio *et al*., 2005). In this regard, alternative techniques, such as the use of a conventional, affordable, digital camera (see below), may provide complementary information, such as the portion of the soil occupied by green biomass, that may help de-confound biomass from the information derived from spectral indices (Casadesús, Biel and Savé, 2005).

Canopy temperature

Because a major role of transpiration is leaf cooling, canopy temperature and its reduction relative to ambient air temperature are an indication of how much transpiration cools the leaves under a demanding environmental load. Higher transpiration represents colder leaves and higher stomatal conductance, both aspects favouring net photosynthesis and crop duration. Relatively lower canopy temperature in drought-stressed crops indicates a relatively better capacity for taking up soil moisture or for maintaining a better plant water status. Thus, higher transpiration is a positive trait when selecting for higher yield potential or better adaptation to mild to moderate stresses. The same may be inferred for higher carbon isotope discrimination when this trait is positively correlated with grain yield. Although canopy temperature may seem very easy to measure, in practice there are methodological problems, particularly in Mediterranean drought environments (Royo *et al.,* 2002; Araus *et al.,* 2002) or where there is not a homogeneous canopy. In fact, screening by canopy temperature measurements under drought stress can be done only during the vegetative growth stage, after full ground cover has been attained, before inflorescence emergence (Lafitte, Blum and Atlin, 2003), at high vapour-pressure deficits in recently irrigated crops and without the presence of wind or clouds (Royo *et al.*, 2005).

Carbon isotope discrimination

Other putative traits, while potentially useful, are less widely accepted, despite being very promising. Such is the case for discrimination against the stable isotope $\Delta^{13}C$, which is limited by the cost of its determination. CSIRO Plant Industry has released recently the two first commercial wheat varieties (cv. Drysdale in 2002, and cv. Rees in 2003) selected for high transpiration efficiency using $\Delta^{13}C$. These varieties are cultivated under rainfed conditions and rely solely upon the precipitation accumulated prior to planting. They have been selected based on their low $\Delta^{13}C$ (and thus high transpiration efficiency), fitting with what has been postulated with regards to this trait. However for Mediterranean environments, $\Delta^{13}C$ (particularly when measured in mature grains) is frequently positively correlated with grain yield (Araus *et al*., 1998a, 2003c; Villegas *et al*., 2000; Condon *et al*., 2004). One of the reasons for this positive relationship is that a genotype exhibiting higher $\Delta^{13}C$ is probably able to maintain a better water status (see Araus *et al*., 2002; Condon *et al*., 2004).

Many other traits, however, cannot yet be recommended as part of an ongoing breeding programme, particularly those that are expensive or difficult to measure. However, some can be used for the selection of parents. Also, QTLs may be mapped for traits such as root characteristics. Nevertheless, the relationship between these loci and drought resistance is not well established (Mackill, Fukai and Blum, 2003). For example, other traits, such as chlorophyll fluorescence for trait evaluation, have long been proposed (Baker and Rosenqvist, 2004), but their application in the field may be strongly affected by crop phenology (Araus *et al.,* 1998b).

18.3.3 How to measure those traits inexpensively?

Carbon isotope discrimination

Given the relatively high costs associated with carbon isotopic analysis (about € 10 per sample), several surrogate approaches have been proposed that are much cheaper, faster and easier to handle. The option most studied has been to use the mineral, or simply the total, ash content of leaves (Masle, Farquhar and Wong, 1992; Mayland *et al.*, 1993; Araus *et al.,* 1998a) or grains (Febrero *et al.*, 1994; Araus *et al.*, 1998a; Voltas *et al.*, 1998). Another promising alternative relies on the estimation of $\Delta^{13}C$ through the Near Infrared Spectroscopy (NIRS) technique (Clark *et al.,* 1995; Ferrio *et al.*, 2001), which carries with it the further advantage of being non-destructive.

Leaf colour

Leaf colour is extensively used due to the speed and ease of use of portable chlorophyll meters (such as the Minolta SPAD™), as well as for the physiological significance of the trait itself. Total chlorophyll content has been extensively used for managing nitrogen fertilization. It provides a high quality, standardized tool for nitrogen management, measured at anthesis in drought-stressed Mediterranean environments. It may be also useful for the screening of the protein content of wheat grains (Rharrabti *et al*., 2001). Moreover, since it is an indicator of early senescence, it has been reported to be positively correlated with wheat yield (Araus *et al*., 1997; Rharrabti *et al*., 2001), and indeed SPAD measurements are routinely taken in breeding programmes. However the cost of a portable chlorophyll meter (at best, at least € 1000) makes this device unaffordable for many breeding programmes in developing countries. This is why IRRI, in collaboration

with NARS, has a multi-panel leaf colour chart developed and calibrated for use with rice throughout Asia (Shukla *et al.*, 2004; IRRI, 2005).

Biomass assessment

Field spectroradiometers able to measure the spectrum of light reflected by the canopy have been expensive devices (exceeding € 12 000). However, the situation is changing. Designed initially for nitrogen management, a simple and easy-to-handle spectroradiometer, such as GreenSeeker™, has become a potentially very useful device for breeding (Figure 18.6). It gives only the basic spectroradiometric indices of green biomass, such as NDVI (as well as the inverse of the SR). Nevertheless, as noted above, these single indices are in fact the most useful for routine breeding purposes. Moreover, as the GreenSeeker includes its own radiation source, it may be used independently of the atmospheric conditions (sunny or cloudy day) and, more importantly, its cost is comparable (or even less) that of a SPAD (below € 3 000).

Conventional digital cameras are low cost devices that could be adopted for generalized use in a number of agricultural applications, including plant breeding. Through adequate processing of the information contained in digital pictures, it is possible to evaluate total green biomass much more cost effectively than with land-based portable spectroradiometers. In addition, digital pictures may also provide information that is not currently acquired through spectral reflectance measurements,

FIGURE 18.6
An example of cheap, easy-to handle spectroradiometer: the GreenSeeker. Evaluation of dry weight of pot plants

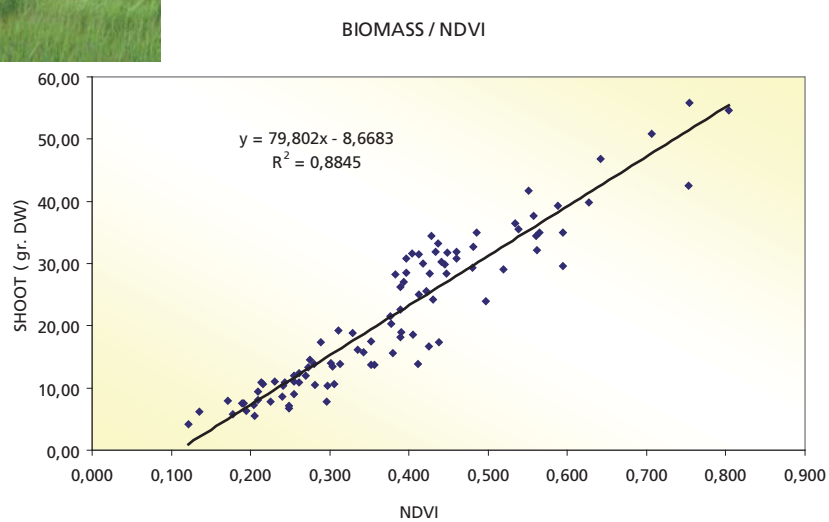
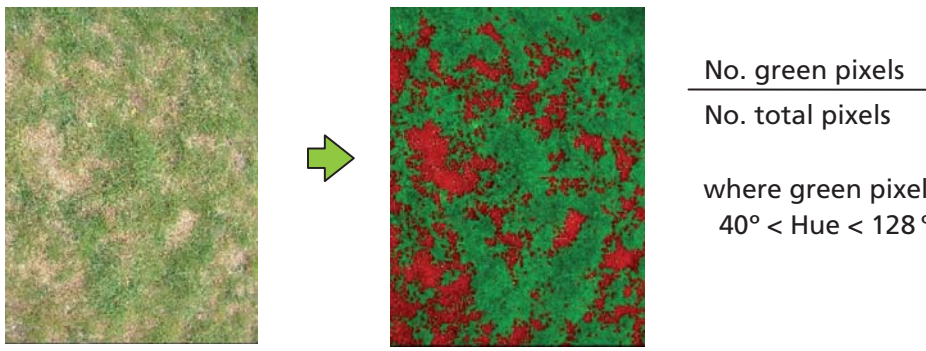


FIGURE 18.7
Some applications of digital photography: Ratio of green area to total area. Easy-to-calculate estimator of green cover



Courtesy of Dr J. Casadesús.

such as the degree of soil covered by the crop (Figure 18.7), the proportion of yellow leaves, or even yield components such as the number of spikes per unit land area (Casadesús, Biel and Savé, 2005).

18.3.4 When to use the traits?

Grain yield is the primary trait for selection in breeding programmes aimed at both increasing yield potential and adaptation to stress-prone environments. When breeding for drought adaptation, the conceptual model used considers yield under drought to be a function of: (i) yield potential; (ii) the flowering date (which indicates whether the crop will avoid drought stress); and (iii) traits that provide drought resistance.

Most breeders select strongly for traits other than yield in the early segregating generations, and do yield testing only at later stages, when a certain level of homozygosity has been achieved and sufficiently large seed quantities are available. While acknowledging the importance of secondary traits, most breeding programmes are not able to integrate them into their selection schemes for the reasons mentioned previously. The decision to advance or reject a genotype is often complex, and in practical terms breeders most often use a system of multiple cut offs. The usual approach is to carry out the selection of early generations in stress-free environments, in order to optimize the expression of desired traits in the plant and simultaneously maximize heritability and response to selection. In early generations, breeders select genotypes that presumably achieve the levels required for the primary traits evaluated in segregating populations (resistance to diseases, plant type, plant height, growth cycle, spike fertility, etc.), choosing only those with potential good score expression in the next generation, when they will be assessed again. Quality is frequently evaluated early, at the family level, in order to detect those crosses with desirable characteristics. It may also be worth evaluating in early generations for some additional physiological traits that may give an indication of yield potential or crop adaptation to abiotic stresses.

At subsequent stages, in more advanced generations, multi-site field experiments are

conducted in order to study the adaptation of lines to the target environments. The combination of yield data with data regarding secondary traits in environments ranging from well watered to high stress allows one to ascertain the adaptability of genotypes to a wide range of conditions, thus making possible more reliable decisions. At this level, selection is mostly based on the main goals of the programme, usually focusing on important commercial traits. However, data on secondary traits may be decisive at this stage in interpreting and explaining G×E interactions, mostly when the heritability of the secondary traits is higher than that of yield, and the genetic correlation of these traits with yield in the target environment is high.

Examples of how to implement indirect selection for physiological traits for drought resistance in different cereals are illustrated in Fischer *et al*. (2003)

18.4 BEYOND BREEDING

18.4.1 Social context

Hall's (2001) definition of ideotype has a wider sense than that first proposed by Donald (1968), being a plan of the phenotype of a cultivar that will perform optimally in a specific set of climatic, soil, biotic and socio-cultural conditions. Emphasis on socio-cultural aspects is now accepted as an important part of the concept of ideotype and embraces the concept of the participatory breeding approach.

Broad-based research, including research on socio-economic aspects of cereal production, is needed to characterize cereal ecosystems in terms of people and their environment; to improve understanding of farming systems, indigenous knowledge, and farmers' practices; and to refine the definitions of the kinds of technologies that should be developed. This will require close interaction with target beneficiaries (IRRI, 1996).

As Bänziger *et al* (2000) state in a comprehensive breeding manual for abiotic stress breeding published by CIMMYT, if the variety being developed for improved tolerance to any stress is unacceptable to farmers for other reasons and is not adopted, all the research work invested in that variety will be wasted. It is critically important, therefore, that farmers be involved in the selection and testing process, and that researchers pay careful attention to farmers' views on what constitutes an appropriate and attractive variety under their circumstances. In such a context, farmer participatory plant breeding represents:

- A dialogue between farmers and scientists to solve agricultural problems.
- A way to increase the impact of agricultural research by developing technologies that are more widely adopted.
- A path to more productive, stable, equitable and sustainable agricultural systems.

Abiotic stress and genetic response for adaptation to stress will depend upon the choice of target environment. To that end, farmer participatory breeding emphasizes three aspects: farmers' knowledge, farmers' ability to experiment, and farmer exchange of information and technologies. Thus, breeding programmes should include participatory on-farm trials, managed by farmers, as part of the testing of a new cultivar. This may ensure that selection has been effective, and that progress made at the station will be transferable to the farm level. Participatory trials should be run concurrently with advanced multiple-environment trials. Moreover, testing for grain quality (and other quality attributes of the crop), in consultation with farmers from the target population environments, is cheaper than replicated yield testing. Hence,

quality screening should be carried out before multiple-environment trials, so as to discard those varieties whose quality would be unacceptable to farmers (Atlin, 2003). A comprehensive methodological approach for farmer participatory breeding can be also found in Ceccarelli *et al.* (2000, 2003).

18.4.2 Crop management and sustainability of cropping systems

Breeding is just half of the equation for more productive and sustainable crops, the other half being agronomic management. The progress that has been achieved for grain yield has been the result of combining improved varieties with appropriate crop management strategies (Cooper *et al.,* 2004). Increased demand for staple crops has resulted in the intensification of agriculture all over the developing world, and one of the most serious consequences of this is soil degradation. When soil is no longer part of a natural ecosystem, if not properly managed, its physical and biological properties become degraded and productivity declines. This has been documented, for example, in the rice–wheat systems of the Punjab in South Asia (Timsina and Connor, 2001). Left unchecked, this process eventually leads to soil loss through erosion and problems of chemical imbalance, such as salinity. Water scarcity adds to the problem, and is intensified in poor soils as they lose the capacity to absorb and retain moisture. The key to this downward spiral is the loss of soil organic matter. While breeding can improve the tolerance of cultivars to salinity and reduced moisture, it is not a sustainable solution if soils continue to degrade. Since soil tillage is the principal cause behind the declining levels of organic matter, crop management practices that minimize tillage whilst keeping crop residues on the soil surface result in healthier soils. These practices are known as conservation (or zero) tillage, and are a potent tool for stabilizing and improving soils (Bradford and Peterson, 2000)

Although conservation tillage is not new (Cline and Hendershot, 2002), it is an alternative strategy that agronomists have been promoting recently in Asia and other parts of the world as a means of combating declining crop productivity (Hobbs, Giri and Grace, 1997). Conservation tillage has significant agricultural and environmental benefits (see Bradford and Peterson, 2000). Improved soil health means less erosion and higher productivity, as well as reducing the probability of encroachment of crops into natural ecosystems. Less carbon emissions are produced thanks to the reduced use of fossil fuels, there is less oxidation of soil organic matter, and less burning of crop residues. Other environmental impacts over time may include less pesticide use as a result of suppression of weeds by residues, and more integrated control of insects. Dust levels in the atmosphere are also reduced because crop residues protect the soil from wind and there is less soil disturbance during field operations. The adoption of zero-tillage on 1.3 million hectares of wheat in South Asia has been achieved through the initiatives of the Rice–Wheat Consortium for the Indo-Gangetic Plains (www.rwc.cgiar.org/rwc).

A management approach for increasing input use efficiency in irrigated environments is the cultivation of crops on raised beds, such that soil movement is reduced, resulting in greater water and nitrogen use efficiency as well reduced herbicide use (Sayre and Moreno-Ramos, 1997; Sayre, 2004). Another technology that is likely to have water-saving applications and is very compatible with raised bed cultivation is alternate-furrow irrigation.

It is well established that when plant roots detect a drying of the soil profile, they send chemical signals to their leaves resulting in a reduction in transpiration rate mediated by reduced stomatal conductance (Davies and Zhang, 1991). The result of this response is a decreased growth rate, but an increase in water-use efficiency. This innate drought response mechanism can be exploited by a modification in irrigation strategy, whereby the side from which plants, growing on top of ridges, are irrigated alternately (Davies, Wilkinson and Loveys, 2002). This was tested in furrow-irrigated maize in China and proved to be highly effective in permitting substantially reduced water application while increasing harvest index and thus not significantly reducing yield (Kang *et al.*, 2000). This simple but effective modification in irrigation strategy has clear potential benefits that can probably be adapted to many irrigated cropping systems.

Precision agriculture is also being applied to increase resource use efficiency. For example, agronomists at Oklahoma State University have adapted spectral reflectance sensor technology for use by farmers to precisely calculate the crop requirement for in-season nitrogen application. By measuring an index (NDVI) early in the crop cycle, levels of N in crop can be predicted (www.nue.okstate.edu). The value of this technology has already been demonstrated in Mexican and Ecuadorian farmers' fields in collaboration with CIMMYT scientists, enabling them to apply precise amounts of fertilizer for yield optimization while minimizing N runoff.

This Tillage Revolution, as it is sometimes referred to, is gaining momentum worldwide (MacIlwain, 2004; Nelson, Giles and Gewin, 2004). The adoption of resource-conserving crop management practices opens up new possibilities for plant breeding, specifically targeted to specific features of crop management. For example, it has been shown that tillage practices influence the composition and intensity of mycorrhizal fungi over a range of soil depths. New ideotypes may be better adapted to emergence and growth among crop residues, to soils with higher levels of organic nutrient sources, and to an increased level and diversity of soil fauna and micro-organisms. This potential synergy between breeding and innovative input-use-efficient crop management practices has been referred to as the Doubly Green Revolution (Conway, 1997).

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CHAPTER 19

Marker-assisted selection in theory and practice

Andrew R. Barr

19.1 INTRODUCTION

19.1.2 Definitions and background

Marker-assisted selection (MAS) involves the use of genetic markers to follow regions of the genome that encode specific characteristics of a plant. For example, a marker genetically linked to a disease-resistance locus can be used to predict the presence of the resistant or the susceptible allele. The reliability of the prediction will depend upon the closeness of the genetic linkage. Markers that co-segregate with the target trait are absolutely reliable and can be regarded as diagnostic.

To be effective, the markers must detect a polymorphism between the plants being analysed. The nature of the polymorphism will vary depending on the marker system being used. Initially markers based on protein differences were widely used. These were based on variation in protein size detected by size fractionation electrophoresis or related techniques, or differences in protein charge or iso-electric point. Specific proteins or enzymes could be detected by staining for total proteins or using the enzyme activity to produce or remove a coloured substrate. Iso-electric variants are referred to as isozymes and were, for many years, extremely important markers for specific chromosomes and chromosome regions. They suffered from two major weaknesses:

- They are limited in number and are often difficult to detect or assay. The total number of isozyme loci that can be assayed is generally fewer than 100 in a well characterized species.
- They detect only low levels of polymorphism. The assays are dependent on revealing a shift in protein mobility through either an altered size or altered iso-electric point, involving a change in at least one amino acid in the protein. Therefore only changes in the coding sequence of a limited number of genes can be detected and changes that lead to an amino acid substitution or deletion. In addition, many such changes are not selection neutral but may involve a change in enzyme function that can be detrimental to other characteristics of the plant.

Detection of sequence variation at the DNA level offers several important advantages over protein-based markers. There are essentially an unlimited number of such DNA markers since sequence variation, in the form of single-base changes, insertions and deletions, or large sequence differences, are abundant. Indeed, in many systems where detailed analysis has been carried out, a sequence difference occurs between two sexually compatible individuals at a frequency of greater than one in every three hundred bases. Most of these changes will be have no detectable effect on plant performance and are selection neutral. In addition, a large range of techniques are now available for detecting sequence variation. The available techniques differ in their ability to detect variation, the ease of assay and the number of loci that can be assayed simultaneously. While early work focused around the use of restriction fragment length polymorphisms (RFLPs) detected via Southern Hybridization, polymerase chain reaction (PCR)-based marker systems are now more widely used, in particular microsatellite or simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP). New techniques not requiring gel electrophoresis are gaining in importance. These include mass spectrometric and Taq-Man type assays. As sequence information becomes more widely available for major crop species, we can expect to see alternatives to gel-based

detection systems applied to identify single nucleotide polymorphisms (SNPs).

Marker development and implementation can be divided into the following steps:

- identify parents differing in the trait of interest;
- develop a population of plants segregating for the trait of interest (using doubled haploids, single-seed descent or F_2 populations);
- screen the population for the traits of interest;
- construct linkage maps of the cross, using bulked segregant analysis or genomic fingerprinting;
- identify molecular markers that co-segregate with the trait of interest;
- test the applicability and reliability of the markers in predicting the trait in related families (also referred to as marker validation);
- produce clear and simple protocols for assaying the markers;
- modify breeding strategy to optimize use of MAS relative to alternative selection techniques; and
- implement into the breeding programmes.

19.2 DEVELOPING MOLECULAR MARKERS

The technique used to identify a molecular marker linked to a trait of interest will depend upon the type of trait and the resources and marker systems available. There are several features of the trait that will be important in devising the most efficient marker development strategy. These include:

- the mode of inheritance—whether simple or polygenic;
- the heritability of the trait and the reliability of the alternative (bio-assay, biochemical analysis, etc.); and
- the ease of bio-assay (this may restrict the size of the population that can be assayed).

The process used to decide on the best approach to marker development is summarized in Figure 19.1. There are five fundamental questions to be asked.

1. Is a marker needed? Marker development is slow and expensive, so it must be clear that there will be a real benefit to the breeding programme through access to a molecular marker. This will involve comparing the cost of marker development and use with more traditional screening methods.
2. Is the trait simply inherited or is it controlled by many genes? If the inheritance is simple, then bulked segregant analysis would usually be the preferred method for marker identification. However, if inheritance is controlled by several genes or is strongly influenced by the environment (low heritability), full map construction or linkage disequilibrium mapping would be the best options.
3. Cost of map construction? The shift to automation and high-throughput marker analysis is dramatically reducing the cost and time involved in marker development. For many species, map construction will be sufficiently cheap to replace Bulked Segregant Analysis (BSA).
4. Is one parent critical or a common source of trait? The question relates to the diversity of germplasm available for screening. If there are multiple sources of the desirable trait, linkage disequilibrium mapping offers the options of localizing loci from multiple sources, while full map construction will only allow the genes from one source to be localized.

TABLE 19.1
Comparison of major marker systems

Marker system	Usual loci per assay	DNA amount	Approx. time per assay	Comments
RFLP	1	5.0 g	5 days	Co-dominant, reliable, often low-level polymorphism
SSR	1	0.2 g	5 hours	Co-dominant, reliable, large number of alleles
AFLP	50	0.2 g	1 day	Mostly dominant, reliable, low level of polymorphism but high multiplexing capacity
RAPD	10	0.2 g	5 hours	Dominant, unreliable in some situations
SNP	1	0.05 g	1 to 5 hours	Co-dominant or dominant, very rapid result depending on technology platform. High development cost
DArT	>100, limited only by chip	5 ng	1.5 to 2 days	Dominant, ideal for fingerprinting as many loci generated from single sample

5. Is there access to large-scale phenotyping systems? Linkage disequilibrium mapping is dependent upon the analysis of a large pool of germplasm and will require substantially more marker assays than full map construction. This is most effectively achieved through automation and high-throughput genotyping.

The marker systems that are currently available, and their relative advantages and disadvantages are summarized in Table 19.1.

The data in Table 19.1 represent averages for these marker systems. Although Table 19.1 would suggest that random amplified polymorphic DNA (RAPD) markers are the most efficient, the unreliability of these markers and the inability to transfer them between crosses has greatly limited their use. The full potential of newer systems, such as Diversity Arrays Technology (DarT) (Jaccoud *et al.*, 2001), is as yet untapped.

Essentially five basic systems have been used to develop markers linked to traits of interest. The relative merits of each and their applicability are outlined below.

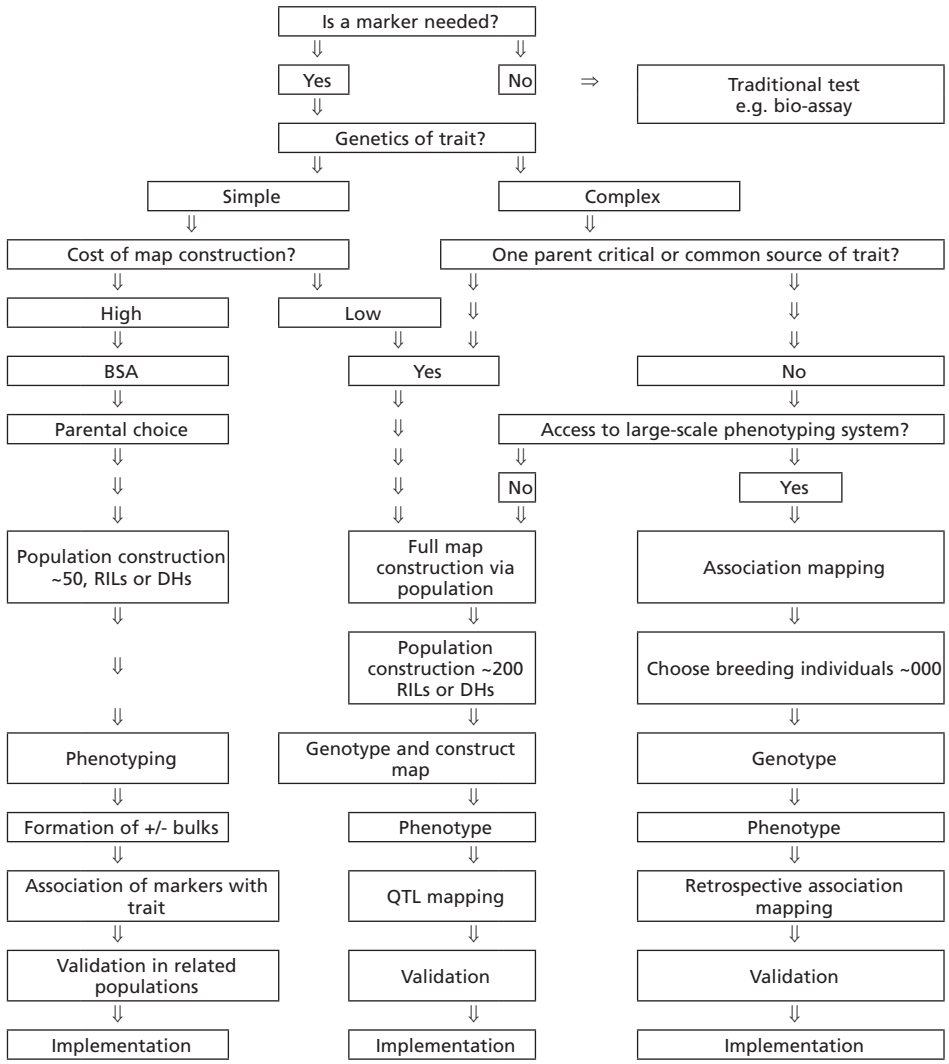
19.2.1 Fully mapped approaches

Complete linkage maps generated from screening the progeny of a cross have provided the basis for most early marker development work. In this process, a population is produced from parents differing in the trait of interest. Molecular markers, able to detect polymorphisms between the two parents, are screened against the population so that each line will have been assayed for the target trait or traits and a large number of markers. Linkage between the markers is assessed based on the segregation pattern. Several public and commercial software packages are available to assist in the map construction. In the first phase of map construction, the markers are divided into linkage groups, usually based on paired comparisons. Markers that fall into a linkage group are then ordered through two- and three-point analysis.

It is often difficult to assign marker-based linkage groups to known chromosomes. For most major crop species, good quality linkage maps have been published. Markers from these maps can be used as reference points to determine chromosome designations. For some species, such as wheat, chromosome substitution, translocation or deletion lines can be used to determine the chromosome designation for specific probes, and hence linkage groups.

With a good quality linkage map, the target trait can be mapped relative to the molecular markers by measuring

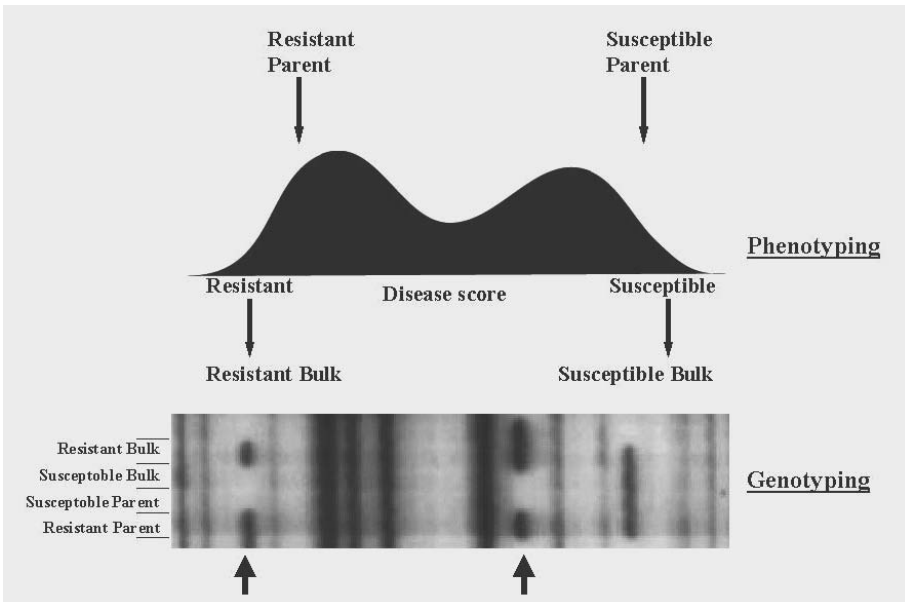
FIGURE 19.1
Marker development strategies



the frequency of trait co-segregation with markers.

Full map construction is difficult and labour intensive, particularly in species with a large number of linkage groups, where linkage maps must be constructed for each chromosome. Usually ten to twenty markers are desirable for each chromosome to give reasonable genome coverage (about 1 every 10 to 20 cM). The work involved in screening this number of markers is considerable if markers such as RFLPs are used. For some cultivated species, the germplasm base may be small and the level of polymorphism between varieties or cultivars low. In order to reveal sufficient

FIGURE 19. 2
Bulked segregant analysis in the identification of AFLP markers linked to a disease resistance locus



Parents, differing in their resistance phenotype, are used to produce a population segregating for resistance. This population is phenotyped for resistance and two bulks are generated: resistant and susceptible. The parental DNA and the DNA pooled from the bulks are analysed with a range of AFLP markers to identify bands present in the resistant parent and the resistant bulk (arrows).

polymorphisms for full genome coverage it may be necessary to screen several thousand probes. Combining RFLP with various other marker systems offers an alternative approach. AFLP markers are particularly useful due to the high multiplexing ratio. Even though the level of polymorphism for AFLP bands is usually lower than for RFLPs, the large number of bands that can be scored in a single assay makes this a valuable marker system for enhancing linkage maps.

It is also often important to consider how many traits of significance may be segregating in the population used for marker development. If several important traits are segregating, the benefits derived from constructing a full linkage map may outweigh the time and costs associated with full map construction relative to Bulked Segregant Analysis (BSA).

Linkage maps of most major crop species have now been constructed, usually based around RFLP markers. These established maps are a valuable resource and a good starting point in identifying the most appropriate markers to use to obtain good genome coverage.

19.2.2 Bulked Segregant Analysis

BSA is a technique that allows rapid, cheap development of markers for simply inherited traits (Michelmore, Paran and Kesseli, 1991). The first step involves pooling individuals from the two phenotypic extremes of a segregating F_2, doubled haploid or similar

population. DNA isolated from the two pools is then screened with DNA markers, usually RAPD or AFLP, and polymorphic bands identified. Clear polymorphisms seen between the two pools will be derived from regions of the genome that are common between the individuals that made up the pools. The remainder of the genome will be randomly contributed by the parents and should show no polymorphisms between the pools. The principle of this technique is illustrated in Figure 19. 2.

This technique has clear advantages for marker identification relative to construction of complete linkage maps. Only a few weeks are required to screen the pools with the AFLP or RAPD primers and the cost will be between 20 percent and 30 percent of that for full map construction. For traits controlled by a single gene, such as many disease resistances, only five to ten plants are required for each bulk, meaning that only small populations are required. However, there are also disadvantages to this technique.

- The method is not effective (although not impossible) for complex traits controlled by several unlinked loci. The technique can be used for traits controlled by fewer than three major loci.
- The actual location of the gene of interest is not revealed, as only linked markers are detected.
- The method can only be efficiently used with PCR-based marker systems such as SSR, RAPD and AFLP markers. For AFLP and RAPD markers, it is usually necessary to convert the marker to a sequenced tagged site (STS) marker, and this is not a simple task. This problem is largely overcome through the use of SSR markers or where very good quality maps are available of AFLP markers. The effort involved in the initial screening of the pools may be greater for SSR markers and a large number will be required, but the benefits in having markers that are immediately applicable in selection programmes may overcome this drawback. Good collections of SSRs are available for most major crop species, but are limited for minor species.

19.2.3 Partial maps

The 'partial map' strategy represents a compromise between the limited resolution of a BSA and the expensive and, in some cases unnecessarily detailed, fully mapped approach. The main advantage of the partial map approach is obviously the cost savings in comparison with full map construction.

As the name suggests, partial maps are constructed of selected regions of the genome. A partial mapping approach is normally applied when some prior knowledge of the genetic control of the trait or gene locations is available, which suggests that a full map is not required for development of the marker–trait association but where the genetic control is too complex for BSA to be successfully used.

Other scenarios where partial maps are useful are where BSA has been used to develop a marker–trait association and consensus maps used to obtain a tentative genomic location for the gene, but where:

- the trait appears closely linked to other traits of interest, and hence linkage relationships are crucial; or
- the marker density on existing maps is too poor for useful markers to be developed.

19.2.4 Pedigree-based analysis

Pedigree-based whole-genome marker development (sometimes called analysis by descent, or similar) is a technique for developing and using marker-assisted

selection in pragmatic breeding programmes by using pedigree information to identify markers linked to traits based on identity by descent in actual breeding populations. This approach therefore bridges the development, validation and implementation stages discussed earlier by making use of the pedigree, phenotypic and genotypic information collected during the day-to-day activities of a breeding programme.

Paull *et al.* (1998) used this approach to develop a marker in wheat linked to stem rust resistance, Jordan *et al.* (1999) identified a marker for sorghum midge resistance, and Eisemann *et al.* (2004), while working with wheat and barley germplasm, proposed an extension to this technique termed 'pedigree-based whole genome marker development', and were developing new software and systems to maximize the information gleaned from such analysis. 'Graphical genotypes' (Young and Tanklsey, 1989) are often integral to pedigree analysis; they are discussed in Section 19.5.2, below.

19.2.5 Linkage disequilibrium mapping

The principle techniques used to identify marker trait associations in crop species have been based around the construction of linkage maps or the use of BSA. Both techniques are based around the production, genotyping and phenotyping of special populations. The populations are generally developed from two varieties that show a major difference in the traits targeted for mapping. This leads to several problems.

- There is usually a high cost associated with phenotyping, particularly for traits requiring extensive field trials or complex analysis, such as many aspects of processing quality and yield. Consequently the number of replicates and of sites are often limited, reducing the sensitivity of some of the analyses.
- The lines (varieties) used to construct the populations are often out-of-date by the time the marker and trait information is available. Many marker development projects for annual crops are using populations that were established five or more years before the marker development work. This reduces the value of the information gathered and the scope of its implementation.
- The structure of the populations limits the types of traits that can be mapped and many of the subtleties of adaptation cannot be analysed.
- Mapping is frequently restricted to known traits for which a well-defined bio-assay is available.
- Validation of the marker is required in populations other than the original mapping population to ensure that the selected allele has a similar effect in different genetic backgrounds.

These limitations in existing mapping strategies can be addressed through association or linkage disequilibrium (LD) mapping. LD mapping is based on seeking associations between phenotype and allele frequencies.

Linkage disequilibrium (see also Chapter 2) is based around the association of marker loci with traits at the population level. Equilibrium is seen in large populations over many generations where there is no selective advantage, or disadvantage, associated with a particular allelic combination in a region of the genome. Disequilibrium appears where selective pressure increases, or decreases, the frequency of particular alleles or allelic combinations. It can be measured through an estimation of changes in allele frequencies as a result of the selective pressure. In this way particular regions of the genome can be associated with particular traits.

There are four potential advantages of this approach in mapping genes in crop species.

- It provides a new perspective for trait mapping, because it uses population structures (based largely around pedigree) and phenotypic data that differ from those used for full map construction of BSA. Consequently, we can expect to see new marker trait associations and targets for more detailed analysis.
- LD mapping also provides detailed fingerprinting information on a large number of lines and varieties and this information will be valuable in several of the breeding strategies outlined below.
- The LD method uses real breeding populations, the material is diverse and relevant and the most important genes (for adaptation, etc.) should be segregating in such populations. The breeder is also integrally involved in the process and this may lead to improved rate and efficiency of validation and adoption. Plant breeders are often reluctant to grow and assess a huge number of lines with little or no potential for direct commercial outcome, as required for complete map construction. The advantage of LD mapping to the breeder is that mapping and commercial variety development is conducted simultaneously.
- Pattern analysis of marker data might detect complex combinations (even epistatic interactions) between alleles at several loci that underlie the superior individuals in a breeding population. This might prove difficult to isolate and validate using the full mapping approach.

Molecular markers offer an easily quantifiable measure of genetic variation within crop species. Many crop species are based on a narrow germplasm pool and display a low level of polymorphism between cultivars. This has hampered the identification of molecular markers linked to agronomically important traits, complicating the differentiation of varieties and the analysis of genetic variability.

19.3 IMPLEMENTING MAS IN BREEDING PROGRAMMES

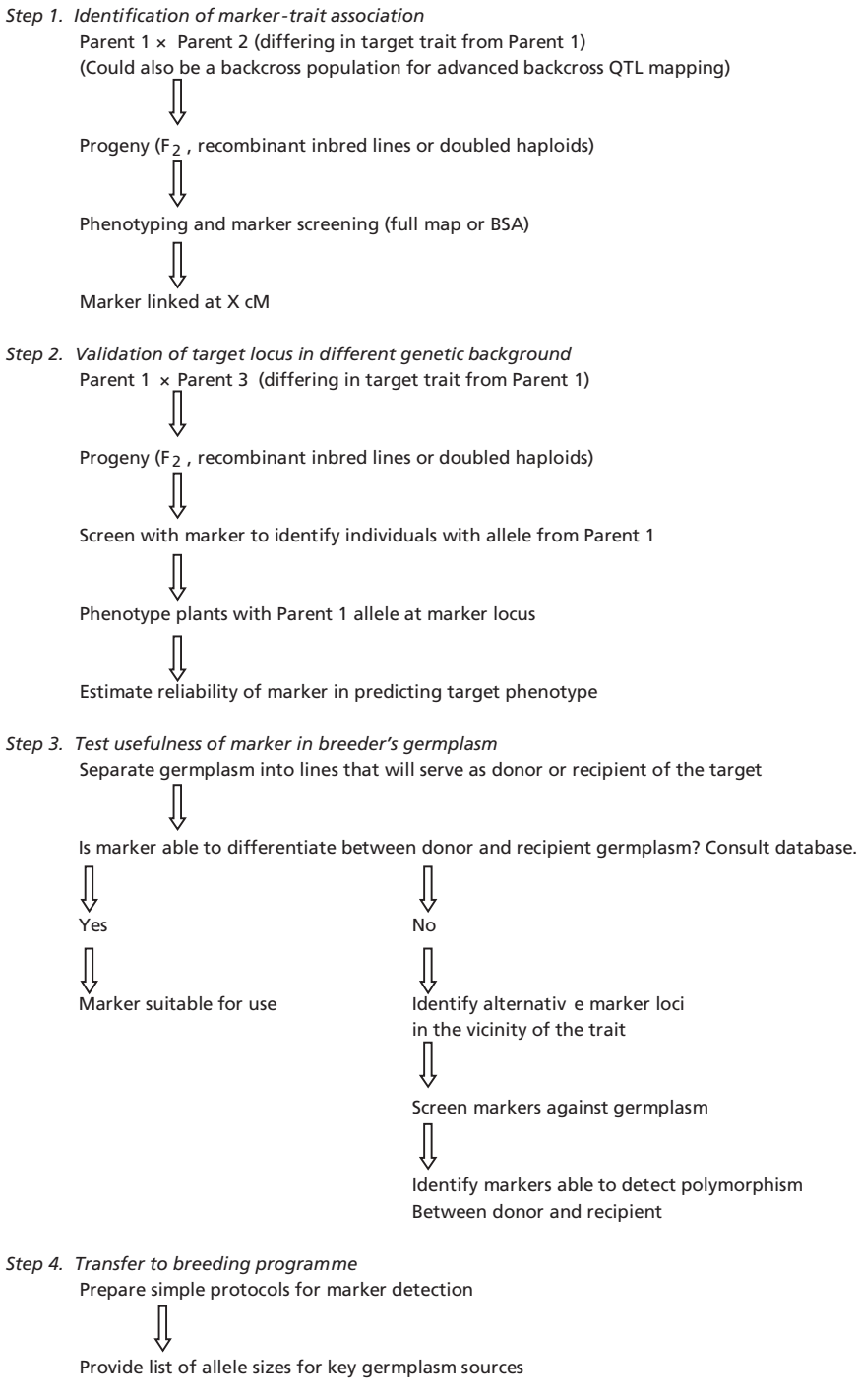
The identification of markers associated with a trait of importance represents a first step in marker application. Several further steps are needed before a marker can be used in a practical breeding programme (outlined in Figure 19.3). The key element is marker validation.

19.3.1 Marker validation

Irrespective of the technique used to identify a marker linked to a target trait, the association has been found in a particular set of circumstances, usually in the progeny of a specific cross (in fully mapped, BSA, pedigree analysis and partial mapped methods). The reliability of the marker can be estimated from the closeness of linkage. As the next step, the ability of the marker to predict the target phenotype must be tested in further populations. Usually one would aim to keep one parent in common in the first test. Therefore, the predictive value of the marker in identifying the phenotype of the plant can be estimated and compared with the original mapping result.

The usefulness of the marker in screening and assaying germplasm in use in the breeding programme is assessed as the next step. The parents likely to be used in the marker screening programme are screened for polymorphisms between those that have and do not have the target trait. Given the narrow germplasm base of many crop plants, a high proportion of markers will fail

FIGURE 19.3
Development, validation and implementation of markers for MAS schemes



this test of applicability. It is then necessary to see if other markers can be found in the vicinity of the target locus that could be used in screening. Ideally one would aim to have about ten marker loci within 10 cM of the target locus to have a reasonable chance of being able to track the locus in diverse germplasm. At this point, markers such as SSRs are particularly valuable, since they tend to detect a greater level of diversity than RFLP-based marker systems.

Finally, a clear protocol for use of the markers must be provided. This will include the following information:

- which molecular marker to use for a given germplasm combination;
- the protocol for the marker assay and the expected size of the alleles;
- the reliability of the marker in terms of the actual success rate of the assay. For example, SSR markers can vary in their ease of assay, with some working almost 100 percent of the time and others showing less than 80 percent success rate. This is often due to the sequence of the primers; and
- the closeness of linkage. This provides a measure of the success of the marker in predicting the target locus or allele.

19.3.2 Structure and function of marker implementation laboratories

Many marker implementation laboratories have evolved from molecular biology research laboratories, and were therefore not well prepared for the demands of achieving regular, high-throughput marker results for demanding plant breeders. Alternatively, some of the newer marker labs have been added *de novo* to plant breeding facilities and may lack the technical backup of adjacent molecular biologists. In either scenario, many marker labs have had difficult gestation periods before they satisfactorily service their plant-breeder clients.

Several crucial factors are required to achieve success in marker implementation labs:

- the plant breeders should be involved in the management and day-to-day activities of the marker lab;
- staff should be selected with technical skill to develop and implement markers, but also with a 'service ethic' that recognizes the importance of timeliness and accuracy;
- the target throughput over time needs to be identified so that the optimum technology platforms can be applied;
- a capital budget is needed that allows for upgrades in the marker platforms;
- the logistics of the entire process needs to be considered, from growing the plants in easy-to-sample, and subsequently cull, containers (often with bar code identification), rapid and accurate label generation for plant and DNA samples, and data management;
- a bio-informatics capability is needed that manages the plants, plant samples, DNA, marker data and selection decisions, and is compatible with the plant breeder's software; and
- access to research molecular biologists should be available for problem solving and rapid recognition of worthwhile new technology.

19.3.3 Marker types

Markers need to be easy and cheap to use. The first widely used marker system was based on RFLPs. They were expensive and technically demanding to apply to many species, particularly for polyploid species or those with a large genome size, such as wheat or onion. Emphasis then shifted to microsatellite markers and AFLPs—

both PCR-based assay systems—that are cheaper and more easily used than RFLPs. The protocols for various marker systems and the pros and cons of the options are widely discussed in the literature and so are not dealt with here (see e.g. Higgins *et al*., 2003; Russell *et al.*, 1997).

Linked markers

Most of the markers deployed in plant breeding at present are linked to the trait of interest, and alleles are detected via polymorphism in non-functional DNA, not variation within the functional genes. This places inherent limitations on the use of the marker screening data. The need to test all parents in the current crossing block to determine the allele associated with the desired genotype has already been discussed. However, it is also necessary to modify the population size of the selected group to allow for recombination between the marker locus and the functional gene.

Diagnostic markers (sometimes called 'perfect markers')

The attraction of diagnostic markers (where the marker is derived from the actual functional gene sequence or shows no recombination with the target gene) is almost overwhelming, particularly when coupled with a simple analysis system, such as SNP. It may be feasible to delete (or at least greatly reduce) the time consuming validation and parental polymorphism stages, and increase the precision of marker assays. However, the costs of developing such markers, while obviously not a limitation for the human genome projects, may be more prohibitive for many crops.

Detailed studies of the structure of key candidate genes, and the characterization of alternative alleles, are generating a new class of diagnostic molecular markers. Increasingly, significant polymorphisms are being identified within the introns of structural genes. These allelic markers may consist of insertion or deletion events, or polymorphic SSR, which are both amenable to gel-based detection systems currently utilized for molecular plant breeding. These markers offer the obvious advantage of not recombining with the gene of interest, and also save implementation resources in establishing parental polymorphisms.

As the research focus shifts from traditional QTL mapping to trait dissection and functional genomics, further candidate genes for MAS will be identified. Coupled with the broad interest in allele mining to identify functional genetic variation, identification of further diagnostic markers is expected.

19.3.4 Polymorphic markers

Mapping a QTL or gene is often only a start for the implementation of a successful MAS scheme. In many situations, several or many markers around the locus of interest are required because of lack of polymorphism among the parents used in the breeding programme. That is, the desired allele of the target is not always linked with the same allele at the marker locus.

Looking at Table 19.2, for a hypothetical cross of Variety 1 × Variety 2, MAS will be effective using either Marker1 or Marker2 as the desired "R" allele is associated with either the 'A' allele at Marker1 or the 'b' allele at Marker2. For the hypothetical cross Variety 2 × Variety 3, there is no polymorphism available between the parents at the marker2 locus, so MAS would have be based on selection at the marker1 locus where the A allele is linked to the desired R allele. For the cross Variety 1 × Variety 4, no polymorphism is available at either of the known marker loci, and so a new marker must be identified. As the number of parental

TABLE 19.2
Hypothetical parent and marker genotypes demonstrating the importance of 'marker polymorphism' in MAS schemes (see text for explanation)

Line	Genotype at Marker locus 1	Genotype at Marker locus 2	Genotype at target gene locus
Variety 1	A	b	R
Variety 2	a	B	r
Variety 3	A	B	R
Variety 4	A	b	r

genotypes increases, so too does the potential problem of lack of polymorphism, and for many breeding programmes this problem has severely curtailed the use of MAS by:

- reducing the number of crosses where MAS can be applied; and
- limiting the complexity of crossing schemes where MAS can be applied. Thus, 4-way crossing plans or convergent schemes are often difficult for MAS application due to lack of polymorphism among parents.

As mapping, QTL analysis, genomics and marker development progress, the number of markers has increased, making this problem potentially less serious in the future for the major crops. Similarly, diagnostic markers render this problem redundant.

19.3.5 Modification to breeding strategies

The power of marker-assisted breeding technologies makes it a very attractive technology for breeders. However, breeders who have been using markers for a long period make the following observations:

- Some parents and some traits do not suit MAS, due to lack of polymorphism between common parents.
- No markers may be available for one QTL contributing to the expression of a particular trait, but perhaps there are for other QTLs affecting the trait.

One extreme consequence is a risk that only parents and populations that suit MAS are constructed and selected. Hence, one must ask "Is the risk of skewing the breeding population greater than the potential gains in efficiency by using MAS?"

Computer simulations have shown that MAS is more effective than phenotypic selection when population sizes are large and heritability is low (Whittaker, Haley and Thompson, 1997; Lande and Thompson, 1990). Modelling also indicates that the combination of genotypic selection (i.e. MAS) and phenotypic selection can be especially powerful. Modification to backcrossing, pedigree and progeny schemes for self-pollinated crops and hybrid breeding to better exploit the power of MAS are discussed in Sections 19.4, 19.5 and 19.9, below.

19.3.6 Intellectual property issues

The use of some marker systems may be constrained by intellectual property protection. Further, research licences may not enable users to apply MAS for selection of commercial varieties without the consummation of a commercial licence. The simplest advice is to ensure you have freedom to operate before embarking on any MAS project.

19.4 USE OF MAS IN BACKCROSS PROGRAMMES

Backcrossing has been widely applied in breeding to transfer a simply inherited trait into an elite cultivar by repeated backcrossing to the elite variety (usually 3 to 6 times) after the initial cross between the donor parent and the elite (or more correctly: recurrent) parent. The gene of interest from the donor parent is usually tracked through the backcrossing stages by using a bio-assay for, say, disease resistance. Selfing is pursued after the backcrossing to fix the genes of interest. Field testing

follows to select the best line from amongst the selfed progeny, usually for similarity to the recurrent parent, expression of the transferred trait and sometimes for improved performance. This process is sometimes called defect elimination, especially when an otherwise elite cultivar has an obvious flaw, which can be rectified by backcrossing.

Breeders like this technique because of its relatively predictable outcomes, yet it is widely viewed as a very conservative breeding strategy and hence many breeding programmes do not allocate a large percentage of their resources to backcrossing. Further, breeders criticize backcrossing for other reasons:

- more rapid genetic improvement is possible by conventional pedigree or progeny methods of breeding;
- there may be no trait(s) of sufficient importance to warrant a dedicated backcross programme;
- an absence of a phenotypic selection system suited to rapid identification of single plants during the backcrossing phase;
- an absence of elite varieties that warrant 'defect elimination' or trait enhancement;
- a long development time; or
- there is little prospect of simultaneous improvement in more than a single trait.

However, molecular markers can benefit a backcross breeding strategy in many ways and turn it from a conservative strategy improving only one trait at a time, to a more aggressive strategy where many traits are simultaneously improved while retaining favoured linkage blocks. The benefits of MAS in backcrossing will be examined, first in general (the following paragraph) and in detail in the seven sections that follow.

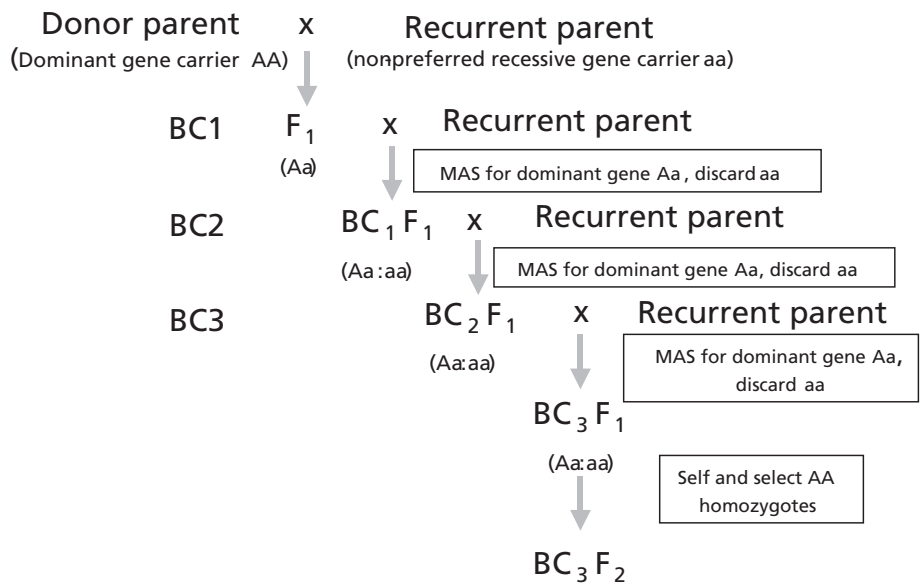
The marker genotype of an individual plant can be determined at very early development stages and therefore plants carrying the gene or genes of interest can be identified prior to flowering and backcrossed once they begin flowering. Up to three backcrosses can be made in this way in one season and plants can be grown in optimum conditions for cross-pollination. Some molecular markers, such as RFLPs, show a co-dominant mode of inheritance and therefore heterozygous individuals can be identified. This is of particular benefit when introgressing a recessive gene. Molecular marker-assisted selection is based on genotype (not phenotype) and therefore is not subject to environmental variation and lower assay error. Molecular markers can be used for selecting regions of the recurrent parent genome unlinked to the introgressed region. This reduces the number of backcross generations required to recover the recurrent parent genotype and increases the probability of obtaining a suitable introgression product.

19.4.1 Transfer of a single dominant gene

This is perhaps the simplest MAS application attempted by most breeders. Here, MAS is used to follow the gene of interest through the backcrossing phase (Figure 19.4) and may directly substitute for a bio-assay (such as a disease resistance test) or a chemical assay (such as an isozyme test). A co-dominant marker is used to distinguish the heterozygous carriers (*Aa* genotype) from the non-carriers (*aa* genotype) during backcrossing generations, to ensure that the gene of interest is not lost before the fixation (inbreeding) stage.

The beauty of the marker test here is that the plants can be sampled at the 1- to 2-leaf stage and a result is available

FIGURE 19.4
Application of MAS to transfer a single dominant gene (Genetic structure of each generation shown in brackets; MAS action shown in text box)



within 5 days, ensuring that the carriers are quickly identified, perhaps re-potted, fertilized and transferred to ideal conditions for crossing when flowering occurs. In contrast, some bio-assays (such as nematode resistance) may be so slow that the plants will have already flowered and crossing must already have taken place before the result is available, meaning that crosses made to non-carrier plants are discarded and represent a waste of effort.

Beckmann and Soller (1986) showed the frequency of the favourable allele was substantially increased where MAS was applied during backcrossing. Care should be taken to ensure that the number of individuals selected for each backcross generation allows for recombination between the marker and the gene of interest, so that false positives do not represent a risk to success.

19.4.2 Transfer of a single recessive gene

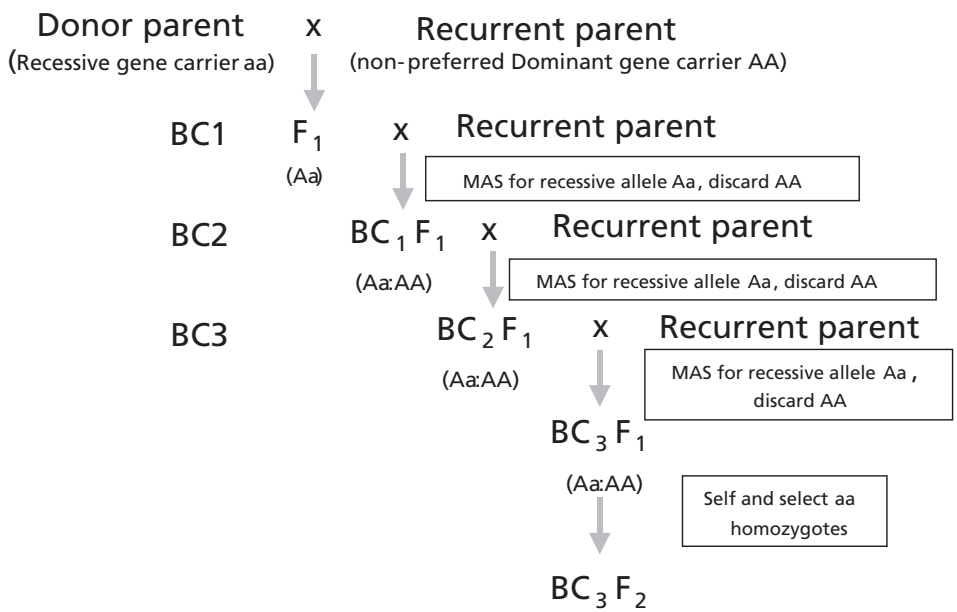
When backcrossing a recessive trait using a phenotypic screening system, a generation of selfing is required in between each backcross to expose the carriers of the recessive gene of interest; in contrast, where a co-dominant marker is available to distinguish the Aa genotype from the AA genotype (Figure 19.5), backcrossing can proceed at the same speed as for a dominant gene.

Hence, it is possible to save the selfing step required between cycles of phenotypic selection in a conventional backcrossing programme.

19.4.3 Selection of several genes simultaneously

One of the most exciting applications of MAS in backcrossing is the potential to transfer multiple traits. It is difficult to

FIGURE 19.5
Application of MAS to transfer a single recessive gene (Genetic structure of each generation shown in brackets; MAS action shown in text box)



conceive and manage practical backcrossing strategies using phenotypic selection to simultaneously transfer two traits, let alone more. In contrast, the application of MAS is only limited by handling an appropriate $BC_n \times F_1$ population to ensure recovery of the required number of individuals heterozygous for all target loci. Hence, quite complex and ambitious defect elimination strategies can be developed and pursued. This may involve introgression of several traits from a single donor parent or simultaneous defect elimination streams with different donor parents (but the same recurrent parent), which are subsequently merged to achieve the transfer of all the targeted traits.

Conceptually, this approach is quite powerful, but in practice the difficulties encountered with this approach have been:

- availability of polymorphic markers as the number of donor parents increases, although this difficulty could be reduced by fixing genes in each backcross stream, by selfing or doubled-haploid production, prior to intercrossing lines from each stream; and
- managing populations of an appropriate size.

19.4.4 Transfer of QTL

Backcrossing has traditionally not been the method of choice for the introgression of quantitative characters into breeding populations. It is often assumed that many genes with small additive effects condition quantitative traits and therefore the probability of recovering a satisfactory number of these genes through backcrossing is very small. While there have been a number of reports of success, most attempts failed to achieve full expression of the donor parent trait. Success appears to be

largely dependent on the number of genes controlling the trait, the heritability of the trait, the ease and timing (generation) of selection, and the desired level of recovery of the recurrent parent phenotype.

QTL marker-assisted selection has the potential to greatly enhance the efficiency of quantitative trait introgression, particularly those derived from exotic germplasm. In conventional selection for a trait of low heritability (most quantitative traits), an individual's phenotype is more greatly influenced by non-genetic factors, such as environment. For a mapped QTL, the phenotypic effect is estimated from the data on many individuals; thus, the influence of non-genetic factors should be reduced substantially (Paterson *et al*., 1991). Therefore genotypic selection for quantitative traits should be more efficient than phenotypic selection so long as a large proportion of the additive genetic variance is associated with the marker loci. In a backcrossing programme, the gains in efficiency are not solely restricted to reduction in selection error, but relate also to logistics and total turnover time. Backcrossing is often the most appropriate breeding strategy for quantitative traits controlled by a relatively small number of loci. As the number of loci increases, the number of backcross individuals that must be grown to have a high probability of recovering each marker allele at all loci increases to a point where backcrossing becomes inefficient.

In its simplest form, there is little difference between the backcross strategy required to transfer a QTL and that for a major gene. Nevertheless, there are important differences. First, there is ambiguity about the location of the QTL, often making the use of flanking markers necessary. This usually increases the size of the introgressed segment, unless the region of interest is finely mapped. Second, unlike a single major gene such as resistance to a nematode, where phenotypic selection during backcrossing is an alternative to MAS, the only way of selecting, for example, a complex grain quality trait is with genotypic selection using markers. It is not possible to test many aspects of grain quality on single plants grown in controlled conditions. Another issue important to QTL transfer is accuracy of selection, and recent studies show MAS significantly improved the accuracy of selection for traits of low heritability. Finally, the most important issue becomes validation of the QTL. This process involves testing the phenotypic effect of the particular chromosomal region derived from a mapping population initially in a range of related germplasm, and then in progressively less-related germplasm.

19.4.5 Selection of donor parent

Many authors have now published phylograms for varieties and genotypes in germplasm pools for major crops in many different agro-ecological zones. A similarity index based on marker data can then be used to select from among a number of possible donors for a desired trait, where the ideal is the minimum genetic distance from the proposed recurrent parent. This will theoretically reduce the number of backcrosses required to recover the recurrent parent phenotype.

19.4.6 Recovery of recurrent parent genotype

In a backcrossing programme, the donor parent genome proportion decreases with an increasing number of backcrosses. As the proportion of donor parent genome decreases, so also should the contribution of the donor parent to phenotypic expression. Therefore, if the proportion of donor

parent genome varies amongst individuals in the same backcross generation, then selection for those individuals will greatly improve the efficiency of the backcrossing programme.

In some species, the range in the percentage of donor parent marker alleles in BC_1 progenies was 8–60 percent, compared with the expectation of 25 percent. It would therefore be possible to save 1 or 2 backcrosses by using MAS to select for a high frequency of recurrent parent alleles, speeding the recovery of the background genotype.

Hospital and Charcosse (1992) and Openshaw, Jarboe and Bevis (1994) used simulations to compare the recovery of recurrent parent genotype following various backcrossing schemes in maize. These studies have further refined the strategies required to optimize backcrossing. Stam and Zeven (1981) estimated that the typical segment length of donor parent DNA retained after three backcrosses was 51 cM in a 100 cM chromosome. Frisch, Bohn and Melchinger (1999) showed that heavy selection in the BC_1 in a simulated maize system was essential to reduce the size of the donor segment, along with tightly linked flanking markers. Frisch, Bohn and Melchinger and Hospital and Charcosse both recommend two to three markers per 100 cM, distributed across the remainder of the genome, for rapid recovery of the donor parent. Frisch, Bohn and Melchinger (1999) are confident that reductions of 2 to 4 backcrosses in a 6-backcross strategy are possible.

AFLPs are well suited to this purpose, subject to an appropriate technology-use licence. SSR-based protocols are also ideal and, depending on source, may be less constrained by intellectual property restrictions. DarT technology may also have application in this area.

The minimization of the length of the intact chromosomal segment of donor type carried (or 'dragged') along with the target gene ('linkage drag') can be achieved by selecting for individuals that are heterozygous at the target locus and homozygous for recurrent parent alleles at two markers flanking the target locus. The estimation of the population size required to achieve this for various flanking marker scenarios has been made in a computer model called Popmin (Decoux and Hospital, 2002), further enhancing the sophistication and precision of the backcross strategy.

19.4.7 Accuracy of MAS during backcrossing

The next general consideration is the accuracy of MAS during backcrossing, which will be determined by:

- the genetic distance between the marker locus and the functional gene; and
- the number of markers available near the gene of interest.

For each backcross progeny selected, the probability of losing the target allele by recombination when selection is performed on a linked marker locus is simply equal to r, the recombination frequency between the marker and target loci. If this is continued for t generations of backcrossing, the probability of losing the target allele by recombination is $1 - (1 - r)^t$. Holland (2004) provides the following examples:

> *If the marker locus exhibits 10 percent recombination with the target gene, there is a 10 percent chance of losing the target allele each generation, and a 27 percent chance of losing the target allele after three generations of backcrossing. Tightly linked markers, of course, do much better: three generations of backcrossing and selection on a marker locus with 1 percent recombination*

with the target gene has only a 3 percent chance of losing the target allele.

If tightly linked markers are not available or you wish to make certain that the gene of interest in included within the introgressed segment, flanking markers can be used. Again, Holland (2004) provides a worked example:

If marker loci A and B flank the target locus, one would select backcross progeny that have both A and B alleles from the donor parent. The probability of losing the target allele with flanking marker selection is equal to the probability of selecting a double recombinant progeny from among the doubly heterozygous backcross progeny. If the flanking loci have recombination frequencies r_A and r_B, respectively, with the target locus, the probability of losing the target allele due to double crossovers within the selected region (ignoring crossover interference) is: $r_A . r_{B/(1-} r_A - r_{B+2} r_A r_{B)}$. This probability can be much lower than the probability of losing the target allele based on selection for a single marker locus. For example, if the flanking markers each have 10 percent recombination frequency with the target locus, there is only a 1.2 percent chance of losing the target allele after a single generation. In any case, with tighter linkage, the chance of losing the target allele and the amount of linkage drag are reduced.

19.4.8 Backcrossing in cross-pollinated species

Many of the opportunities and roles of MAS in backcrossing in cross-pollinated and hybrid crops are similar to those covered earlier in Section 19.4. One subtle difference is that for crops such as maize, where a great deal of information about the genetic control of characters is known, it may be more common to transfer 1 to 5 chromosomal segments associated with the expression of a semi-quantitative trait than may be the case in other crops (Stuber, Polacco and Senior, 1999).

19.4.9 Summary of backcross breeding using MAS

The advent of molecular marker technology has dramatically increased the potential efficiency, precision and flexibility of backcross breeding. However, these gains are yet to be realized by most practical breeding programmes. The first commercial varieties selected from backcrossing enhanced by MAS have now been commercialized, and more will follow shortly. The challenge is now for new crops and breeders to realize the potential of the increased power of backcrossing.

19.5 USE OF MAS IN PEDIGREE OR PROGENY BREEDING

In many breeding programmes, backcrossing accounts for only a small percentage of the total germplasm. While improvements in backcross efficiency are important, the biggest potential impact of MAS in most programmes for self pollinated plants lies in pedigree or progeny breeding systems, based on so-called 'forward crossing' strategies (Holland, 2004).

For perennials, and especially long-lived perennials, which take years to reach their reproductive stage, markers are especially valuable. For instance, if marker+trait associations for fruit quality in stone fruits or pulp yield in forest trees were available, the impact of markers would be especially high, as only those seedlings with some chance of success need to be progressed to field trials, greatly increasing the cost-effectiveness of the breeding work.

In the following sections, the important issues and opportunities in the application of MAS in pedigree and progeny breeding schemes are discussed.

19.5.1 Characterization of the germplasm pool

The first attempt of many breeding programmes to utilize molecular markers in their wider breeding programme is often the characterization of the genetic diversity of their germplasm pool (e.g. Melchinger *et al.*, 1994). Markers are chosen that have wide genomic coverage (even better if hotspots of specific interest within the genome are known), which are then assayed over key representatives of the germplasm base of the programme, including significant ancestral material, successful parents, representative genotypes from other major improvement programmes in the world, and current elite varieties.

This information can be used to:

- choose donor parents closely related to recurrent parents for backcrossing, as described above;
- choose parents with wide genetic diversity to maximize opportunities for transgressive segregation for traits of interest or to define possible 'heterotic groups'; or
- map traits based on pedigree and marker similarity, as demonstrated by Paull *et al.* (1998) for Sr 22 in wheat. Forster *et al.* (1997) demonstrated AFLP associations with salt tolerance within *Hordeum spontaneum* germplasm.

19.5.2 Linkage block analysis and selection

Breeders have long suspected that certain chromosomal regions carry critical clusters of genes (linkage blocks) that have been highly conserved during selection. The term 'national parks' has been coined to describe these linkage blocks. Few breeders have attempted to characterize linkage blocks in the breeding material and most do not have the tools to exploit this information. However, such linkage blocks have now been characterized using molecular markers in many crops, including barley and maize. The usual methodology is to establish a genotypic database of genotype × marker loci. This information can be used to develop graphical genotypes, a technique where genotypes of lines are visualized in a range of software applications, which make it easier to interpret complex datasets. These software applications can be used to select individuals that carry alleles closest to an ideal combination, as defined by the linkage block analysis. Currently, the most advanced software for graphic genotypes resides in the private sector, although several programmes are currently available in the public domain, e.g. GGT, Hypergene® and Geneflow®.

19.5.3 Key recombination events

One of the greatest positives to come from marker development programmes has been the increased knowledge of the genome and the physical and genetic control of important traits. After the first decade of research in this area, breeders can feel much more confident of designing an appropriate strategy to transfer a trait from one parent to another, or indeed improve a quantitative trait. Hence, breeding strategies can be modified to:

- recognize coupling and repulsion linkage between key traits;
- ensure that a breeding population is of sufficient size to capture the key QTLs contributing to a quantitative trait; and
- ensure key major genes form the base of each important segregating population

19.5.4 Validation of F_1s

Although most breeders prefer not to acknowledge the problem, up to 5 percent of all crosses made in pragmatic breeding programmes may be incorrect, i.e. they are selfs or encompass incorrect parents. Molecular markers are useful in identifying and eliminating incorrect crosses, and absolutely essential in the production of mapping populations, genetic studies and for crosses destined for doubled-haploid production.

19.5.5 Enrichment of complex cross F_1s

Three- and four-way crosses are attractive since they potentially allow a greater range of desirable traits to be simultaneously incorporated into elite progeny. However, in practice, many breeders find that the frequency of elite progeny is very low. Hence, they prefer to take the longer route of making simpler crosses, fixing desirable alleles and then intercrossing selected lines from each of the simpler crosses. One alternative is to use MAS to increase the desirable allele frequency for each locus contributed from a quarter parent from 25 percent of progeny, to 50 percent by screening the top cross F_1 or four-way cross F_1s. This has proven to be single biggest use of MAS in some programmes, as it has huge leverage on the frequency of desirable alleles across the whole germplasm pool.

Many traits can be screened in complex crosses, but the conundrum posed by this strategy is that although marker technology has made complex crosses more useful, the added complexity may ultimately constrain its application by lack of polymorphism among parents.

19.5.6 Pyramiding genes

A special case worth mentioning is that of 'pyramiding genes', i.e. accumulation of a number of genes affecting the same trait, e.g. to build a resistance mechanism with greater chance of showing durable resistance or gain greater expression of a quantitative trait. The use of molecular markers, and MAS in particular, offers great advantages over classical genetics, as shown in Table 19.3. Not only is the breeder more certain of the number of alleles contributing to the overall pyramid, but the time to release of the new variety is reduced by avoiding the need to dissect the pyramid using testcrosses and progeny testing.

TABLE 19.3
A comparison of building and dissecting resistance gene pyramids using classical versus molecular breeding techniques

Step	Classical breeding	Molecular breeding
Building pyramids	Make cross between different resistant parents	Make cross between different resistant parents
	Select for most highly resistant phenotype	Marker-assisted selection for different contributing loci
	Use 'ultra' virulent races, when available	Applicable to major and minor genes
	Very difficult for genes of small effect	Genotype of final lines firmly established, except for possible recombination between marker use and gene (use flanking markers)
	Genotype of final line may not be clear	
Dissecting pyramids	Retrospective analysis of developed lines required to prove all important alleles are included	No need, as genotype known from marker assay
	Resistant × Susceptible cross made, F_1 to test dominance relationships, F_2 analysis for number of genes	
	Progeny test and/or Test-cross to confirm	

19.5.7 Early generation selection

The selection theory required to implement MAS in early generations is similar to other forms of selection, although MAS is closer to 'simultaneous' rather than 'tandem' (or sequential) selection, which is often a feature of early-generation phenotypic selection. In general, breeders visually select traits of high heritability in early generations because it is not possible to effectively select for yield (and some other complex traits) in rows or small plots. Computer simulations have shown that MAS is more effective than phenotypic selection when population sizes are large and heritability is low (Whittaker, Haley and Thompson, 1997; Lande and Thompson, 1990). This challenges breeders and geneticists to design and implement practical MAS strategies to effectively select complex traits in early generations.

MAS of early-generation fixed lines is therefore the ultimate goal of the many programmes. However, at present, this is fantasy for public-sector breeders, as a number of limitations dictate that MAS can only be used in early-generation screening for very important material, comprising a small fraction of the total programme. The key limitations are the cost of DNA extraction, availability of suitable markers, staff resources for sample and data handling, and the costs (fixed and recurrent) of high-throughput systems.

19.5.8 Trait dissection

The use of QTL analysis to dissect traits is proving a powerful tool. Trait dissection relies on the co-incidence of QTLs derived for different characters, which is then used to infer associations. For instance, in barley, the QTL for a thin husk is co-incident with QTL for malt extract, inferring that malt extract (the economically important trait) is associated with a thin husk. Intuitively this was reasonable, and subsequent genetic and biochemical analysis showed this to be true. Other QTLs for malt extract were co-incident with QTLs controlling levels of starch-degrading enzymes—again, this association was subsequently proven to be causal. While trait dissection may not be as powerful an approach to the resolution of complex characters as expression profiling or functional genomics, it is certainly proving a useful, practical tool in many breeding programmes.

19.5.9 Summary of use of markers in backcrossing and pedigree and progeny breeding systems

In the previous two sections, the applications, opportunities and limitations of MAS in backcrossing and pedigree and progeny breeding systems were discussed. In Table 19.4, these roles are summarized for a typical breeding programme for a self-pollinated crop.

19.6 USE OF MAS IN THE EXPLOITATION OF PRIMITIVE GERMPLASM

Genetic diversity in modern cultivars of some crop species is diminishing due to the high selection pressure for specific quality and performance characteristics. This may ultimately lead to a reduced capacity for breeders to respond to new disease pressures and may restrict breeders from making major improvements in yield and quality parameters. Introgression of germplasm from outside the current gene pool is essential. Such introgressions have typically sought to introduce major genes, such as those for disease resistance, via backcrossing—this type of project has been a long-standing and successful part of many breeding programmes, and it is easy to envisage a role for MAS along the lines discussed earlier.

TABLE 19.4
Summary of roles for MAS in breeding programmes in self-pollinated crops

Phase	Pedigree and Progeny	Backcrossing	Marker roles
Parental choice	A × B		Understand the genetic relationships between current and future members of the germplasm pool. In hybrids, estimate likely heterosis through diversity analysis.
		A × B	Choose parents with small genetic distance to reduce number of backcrosses. Develop strategy for introgressing and selection of "quarantine traits". Transfer transgenes into elite lines
Crossing		BC_1 F_1 × A BC_2 F_1 × A BC_n F_1 × A	Identify progeny Carrier of trait Low percentage donor genome Small introgression segment Carrying domestication traits (for wild × cultivated crosses) Save 1 generation per backcross for recessive traits
	A × B		Check veracity of cross
	(A × B) × C		Enrichment of F_1s – markers used to characterize germplasm
	(A × B) × (C × D)		Enables more complex crossing strategies
Segregating generations	F_2 to F_n		High throughput markers required to select desired alleles Markers used to identify desired progeny in parent building schemes
		Fixation	Use markers to choose lines close to recurrent parent to 'fast-track' line to release by reducing evaluation requirements as before
Evaluation of fixed lines	Year 1 Limited sites, limited replications Year 2 More sites, more replications Year 3 Regional trials Year 4 National list Year 1 Year 5 National list Year 2		Limited role until QTL for adaptation/quality validated
		Year 1 Limited sites & seasons Year 2 Regional trials Year 3 National list	Whole genome marker analysis can identify individuals close to recurrent parent, thereby saving expensive yield and quality testing
Pure seed			Random genome survey plus key economic traits to ensure purity Markers used to compare re-selections for bulking
Commercialization			Use markers to provide evidence in "essentially derived" discussions Markers used to identify or compare new varieties against other varieties of "common knowledge"

However, MAS may be able to do much more. The QTLs for agronomic and quality traits in many programmes are largely from elite breeding lines and cultivars, with relatively little effort currently being invested in the identification of QTLs from outside this elite (and narrow) gene pool. Experience from some crops (e.g.

tomato, barley, wheat), indicate major improvements in quantitative characters can be made through the exploitation of exotic germplasm. However, this is a difficult and time consuming task for most plant species, and presents two major challenges, both of which can be better met with MAS than with conventional tools. These are considered in the following sections.

19.6.1 Backcross strategies to manage domestication traits

The domestication process for annual plants often requires modification of:

- seed dormancy (usually a reduction);
- seed dispersal (improve seed retention for mechanical harvesting);
- uniformity (greater synchrony of flowering to ensure more even ripening); and
- removal of leaf, seed or fruit toxins and anti-nutritional factors.

An example of the domestication process in the latter twentieth century was the conversion of the wild blue lupin (*Lupinus angustifolius*) to a sweet, domestic white lupin used widely for stock feed. While some of the domestication traits were dominant alleles expressed so that visual (phenotypic) selection was possible, seed toxins and dormancy selection was more difficult, with the result available after the opportunity for crossing had passed. More recent attempts to domesticate the related species *L. atlanticus* and *L. pilosus* have been greatly enhanced by use of MAS for the domestication traits, applied to seedling plants so that selected individuals could be crossed in that generation. Where further traits are required from wild germplasm, gene introgression would be greatly facilitated by MAS against all weedy traits during the backcrossing phase.

19.6.2 'AB-QTL' techniques to identify and introgress QTLs

Unadapted or wild-species germplasm has been used mainly as a source of major genes for disease and insect resistance, which can be readily introgressed into adapted types through backcrossing. While it is relatively simple to identify disease resistance in unadapted germplasm, it is difficult to identify accessions that are likely to carry genes for quantitative traits, as the unadapted germplasm is almost always inferior to adapted germplasm for these traits (Tanksley and Nelson, 1996). Several studies in tomatoes have demonstrated that mapped QTLs isolated from wild accessions can substantially improve commercial tomato varieties. Traditional QTL mapping techniques, however, have several deficiencies when applied to unadapted germplasm. These include the high frequency of undesirable genes (e.g. shattering and sterility), making the collection of meaningful data difficult. Also, epistatic interactions are statistically difficult to detect, yet are likely to occur in high frequency in conventional populations (the most desirable QTLs are those not requiring epistatic interactions).

Tanksley and Nelson (1996) proposed that these problems could be surmounted with QTL analysis by delaying analysis to an advanced backcross generation (BC_2 or BC_3). This would overcome agronomic problems associated with individuals within a mapping population carrying a high proportion of wild germplasm. The detection of QTLs with epistatic effects would be much reduced. Deleterious effects due to linkage drag would also be less likely.

The potential of this method to produce commercial cultivars has been successfully demonstrated in tomatoes by Tanksley *et al.* (1996). The potential in other crop species is currently being tested, with the first

papers on wheat, barley and rice just being published. It is likely to be more difficult in those crops with more complex genomes. However, this methodology may facilitate the exploitation of relatively underutilized genetic variation in wild ancestors and related landraces of many crop species.

19.7 USE OF MAS IN PARTICIPATORY PLANT BREEDING STRATEGIES

Participatory plant breeding has been proposed as a useful method of simultaneously achieving genetic progress through plant breeding, as well as facilitating seed production and variety adoption. The technique has special application in resource-poor farming communities. The basic principles of a participatory scheme are that:

- the breeder generates a relevant pool of genetic variability for the target environment;
- the breeder multiplies seed of these segregating populations for distribution to local participators (usually farmers or locally-based agronomists);
- the breeder facilitates trials of these populations;
- the participators identify the single plants or lines they feel are relevant to their agro-ecological zone and end uses;
- seed is multiplied by the breeder, who then facilitates merit trials at local level;
- the participating farmers and breeder jointly select elite lines, which are further multiplied for regional testing and seed production; and
- the participating farmers spread seed of new elite material for commercial production.

Similar schemes are now widely used in the CGIAR network and their full potential has still to be unlocked. MAS may have some useful roles to play in participatory schemes.

19.7.1 Enrichment of germplasm pools

The success of the participatory approach will depend on the frequency of desirable alleles in the segregating populations supplied to the participating farmers. Hence, there is a role for MAS applied to pre-select TC_1F_1 or BC_1F_1 individuals carrying desired alleles, or, if greater marker throughput is possible, individuals could be selected from segregating populations for distribution to the participating farmers.

19.7.2 Pre-breeding for the participating farmers

If the participating farmers in the breeding programme have a set of minimum criteria (such as disease resistance genes, plant development or stature genes, quality traits, etc.), which they would like in a range of adapted backgrounds from an earlier cycle of the participatory breeding process, these could be provided to the breeder for enrichment before return to the participating farmers.

19.7.3 Seed production

A successful participatory programme will produce many lines for commercialization for different, small, regional areas. The task of seed production and maintenance can therefore be complex. Marker fingerprinting of lines could be used to ensure the integrity of seed stocks, both prior to release to the participating farmers and to maintain pure seed in the longer term.

19.8 USE OF MAS IN TRANSGENIC BREEDING PROGRAMMES

MAS has been extremely important in the selection and monitoring of transgenics following the development of transgenic plants. The reluctance of the food, feed and

processing industries to accept transgenic varieties has restricted the application of the technology. However, it is hard to predict shifts that might occur in public attitudes over the next ten to twenty years—the timeframe of a breeding programme. It is therefore important that genetic engineering be included in the planning of breeding methodologies for many crops, in addition to those where commercial transgenic varieties are currently available. The expectation is that many genes will be amenable to manipulation via genetic engineering. The transgenics will need to be closely monitored through the breeding programmes to satisfy regulatory requirements and to ensure their rapid deployment. This will have the effect of increasing the number of loci that need to be tracked and will require breeding strategies that effectively integrate the transgenic screening with other aspects of MAS and breeding targets.

Some companies have combined marker-assisted identification of transgenics with Geographical Positioning Systems (GPS)-based bioinformatics technologies to track and monitor transgenics in field trials. This is often a requirement of various gene technology regulation authorities operating in many countries during the pre-commercial evaluation phase.

Markers also play a crucial role in determining the position, size and number of inserts of the functional transgenics. In addition, markers can be used to track the selectable marker used during transformation and assist in the selection of transgenics free of the selectable marker, post-transformation.

19.9 PRACTICAL APPLICATION OF MARKER-ASSISTED SELECTION IN BREEDING PROGRAMMES

In this section, six examples of practical application of MAS in the South Australian barley breeding programme are described to illustrate some of the principles described in this chapter. The progression of the type and scale of MAS can be followed by looking at papers presented at the International Barley Genetics Symposiums of 1996, 2000 and 2004 (Langridge *et al.*, 1996; Barr *et al.*, 2000, 2004).

19.9.1 Transfer of a single dominant gene

This was the first MAS application attempted by the barley programme and has passed the ultimate test: it resulted in the release of a new variety that has been a commercial success.

Example 1: Transfer of resistance to Cereal cyst nematode into the malting variety Sloop

The transfer of the gene *Ha*2 for resistance to Cereal cyst nematode (CCN) from the feed variety Chebec to the malting quality variety Sloop was initiated in 1994. MAS was applied using the RFLP marker *Xawbma*21 for 3 cycles of backcrossing. Doubled haploid plants were produced from the BC_3F_1 and 66 percent of the regenerants were classed as CCN resistant. This exceeds the expected proportion (48 percent, for a marker 3 cM distal from *Ha*2) as it seems that a region associated with improved regeneration from tissue culture is linked in coupling to the *Ha*2 gene from cv. Chebec. It is estimated that the use of MAS saved at least two years compared with phenotypic selection. The best of the CCN-resistant BC_3-derived Sloop types was released in 2002 and registered under Plant Breeders Rights in Australia as cv. Sloop SA. In 2002, all 200 tonne of breeders' seed was sold to farmers eagerly awaiting the new malting variety with the resistance required to manage CCN infestations in

their cropping rotations. By 2005, it is estimated that nearly 100 000 ha would have been sown to Sloop SA in South Australia.

Example 2: Transfer of resistance to BYDV into the malting variety Sloop

A second example is the successful transfer of Barley Yellow Dwarf Virus (BYDV) tolerance conditioned by the gene *Yd2* from cv. Franklin into cv. Sloop, using MAS with the PCR marker YLM (Paltridge *et al.,* 1998). MAS was employed through two backcross cycles and the BC_2F_2 derived lines were field tested in plots inoculated with viruliferous aphids. *Yd2* was successfully transferred to the BC_2 lines, and losses due to BYDV were dramatically reduced.

19.9.2 Transfer of a single recessive gene

Provided a co-dominant marker (such as a RFLP or SSR) is available, it is possible to avoid the selfing step required between cycles of phenotypic selection in a conventional backcrossing programme. This then makes the transfer of a recessive gene, both conceptually and practically, little different to the above example of a major gene. In barley, an example of a recessive trait with linked markers is the *mlo* gene for resistance to Powdery mildew, which has been transferred to many new lines via MAS during backcrossing.

19.9.3 Selection of several genes simultaneously

One of the most exciting applications of MAS in backcrossing is the potential to transfer multiple traits. It is difficult to conceive and manage practical backcrossing strategies using phenotypic selection to simultaneously transfer two traits, let alone more. In contrast, the application of MAS is only limited by handling an appropriate $BC \times F_1$ population to ensure recovery of the required number of individuals heterozygous for all target loci. In a defect-elimination strategy with the cultivar Sloop, the South Australian Barley Improvement Programme (SABIP) introduced genes for resistance to CCN (either *Ha2* or *Ha4*), tolerance to BYDV (*Yd2*), resistance to Spot form of Net blotch (SFNB) (*Rpt4*) and manganese efficiency (*Mel1*) into cv. Sloop in parallel backcross streams. Beginning in 1996, these independent streams have been progressively merged. Sloop types with combinations of these traits entered field trials in 2000.

The difficulties encountered with this approach have been:

- availability of polymorphic markers as the number of donor parents increases, although this difficulty could be reduced by fixing genes in each backcross stream, by selfing or doubled-haploid production, prior to intercrossing lines from each stream; and
- managing populations of an appropriate size.

Example 3: Backcross conversions — Transfer of resistance to SFNB and CCN to cv. Gairdner malting barley from cv. Keel feed barley

Cv. Gairdner is a widely adapted malting variety bred by Agriculture Western Australia. It has a good disease resistance profile, with resistance to Leaf scald, BYDV tolerance (*Yd2 locus*), Powdery mildew (presumably cv. Franklin's two genes) and net form of Net blotch (unknown), but it is susceptible to Leaf rust, SFNB and CCN. The high yielding, feed variety cv. Keel has resistance to SFNB (*Rpt4* plus another locus (or loci) conditioning adult plant resistance), Leaf scald (unknown) and CCN (*Ha4).*

We designed a fast-track strategy using single-seed descent, which was initiated in 1998, to rapidly introduce resistance to SFNB and CCN from cv. Keel into cv. Gairdner. We used MAS and bio-assays in large populations of the BC_1F_1 and BC_1F_2 to select for *Rpt4* – the gene conferring seedling resistance to SFNB. BC_1F_4 individuals were multiplied over summer and placed into Stage 1 field trials in 2000. Following phenotypic selection for SFNB and CCN resistance and Gairdner plant type, the remaining 43 individuals were tested for *Yd2*, *Rpt4*, *Bmy1* (Gairdner carries the SD1 allele for β-amylase) and three malt extract QTLs (1H, 2HL, 5H). The malt extract QTLs are important to maintain the good malting quality of Gairdner in the face of introgression of potentially undesirable alleles from the feed variety Keel. Four lines were promoted to Stage 4 trials in 2001, based on the marker profile and their field performance in 2000. In this cross, the Gairdner allele for malt extract on 1H was the most significant, providing 2 percent higher extract than the Keel allele. This strategy would have been greatly assisted by markers for CCN resistance (we did not know Keel carried *Ha 4* until 2000) and the 'second' adult plant gene in SFNB resistance. Further, it would have been useful to have access to a wider range of SSR markers and the ability to deploy them cost effectively. While this project started with a BC_1F_1 population of over 150 individuals per generation, there were still too few selected lines by the BC_1F_7. Nevertheless, promising lines completed Stage 4 trials in 2001, with excellent yield, malt quality and disease resistance. Seed (500 kg) was produced of the best 3 lines in 2002 and release was to proceed in 2006, subject to satisfactory performance in commercial malting and brewing trials in 2005.

The next phase in the Gairdner defect elimination is to introduce genes for boron tolerance, leaf rust resistance, leaf scald resistance and the SD3 allele for thermostable β-amylase. The crossing strategy involves merging five streams (Table 19.5).

By the time the seed of this complex cross is available, it is hoped that markers for the 'missing' genes will be available and that it will be in practise possible to select for the Gairdner background genotype using SSR markers. We have avoided using AFLPs for selecting the recurrent parent genotype because of the cost of implementing a commercial licence.

From Table 19.6, we can see the genotypes of the final 22 lines, which were derived from a combination of genotypic (MAS for the loci shown) and phenotypic selection (yield, malt extract, malt diastatic power, and resistance to CCN and SFNB). The graphical genotypes show the effectiveness of MAS for changing the allelic frequency at the target loci, but also show that unselected regions, such as EBMAC501 on chromosome 1H, BMAC310 and large regions of chromosome 7, have more Keel alleles than expected. These regions may carry Keel alleles that affect the traits targeted by phenotypic selection, but are as yet uncharacterized in QTL studies.

TABLE 19.5
Trait donors for five streams of the Gairdner defect elimination programme

CCN + SFNB stream	Boron stream	SD3 stream	Leaf scald stream	Leaf rust stream
Gairdner/Keel// Gairdner	Gairdner/DH115/ / Gairdner	Gairdner/SD3// Gairdner	Novel alleles from *Hordeum spontaneum*	Complex cross of Gairdner and Fanfare

TABLE 19.6

Marker genotype of 22 lines from the cross Gairdner/Keel//Gairdner assessed at 47 marker loci

Chromosome	Primer	cM	WI3584	WI3585	WI3586	WI3587	WI3588	WI3599	WI3600	WI3601	WI3602	WI3603	WI3604	WI3673	WI3674	BX98A061D/8	BX98A061D/12	BX98A061D/13	BX98A061/129	BX98A061/1130671	BX98A061/1130672	BX98A061/1241739	BX98A061/1241747	BX98A061/4515	Keel alleles	Gairdner Alleles
1H	EBMAC501	37	B	A	A	B	B	B	n/r	B	B	A	B	A	A	B	B	A	A	B	B	A	A	B		
	BMAC213	52	A	A/B	A	A	A	A	B	A/B	A	A	A	A	A	A	A	B	A	A	A	A	A	A		
	BMAC032	97.3	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A		
2H	BMAC134	4	B	B	A	B	B	A/B	A	A	B	A	B	A	A	B	A	A	A/B	B	B	A	A	A	Possible sf	
	BMAC881	71	A	A	A	A	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A		Malt extract
	BMAC878	72	A	A/B	A	n/r	B	A	A	A	n/r	A	A	A	A	A	A	A	A	A	A	A	A	A		
	BMAC140	78	A	A/B	A	A/B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A		
	AWBMS56	85	A	A	A	A/B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A		
	BMAC125	89.9	A	A	A	B	A/B	B	A	A	n/r	A	B	A	A	n/r	B	A	A	A	A	A	A	A		
	BMAC114	90	A	A	n/r	A	A	A	A	A	B	A	B	B	A	B	B	A	A	n/r	A	A	A	A		
3H	BMAC209	31	A	A/B	A	A	A	A	A/B	A/B	A	A/B	A	B	B	A	A	A	B	A	A	A	A	A		
	BMAC906	45	A	A	A	A	A	A	A/B	A	A	A	A	B	B	A	A	A	B	A	A	A	A	A		
	BMAC603	45	A	A	A	A	A	A	A	A	A	A	A	B	B	A	A	A	B	A	A	A	A+	A+		
	BMAC112	56	A	A	A	A	A	A	A	A	A	*	A	B	B	A	A	A	A	A	A	A	A	A		Yd2
	HVM060	76	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/B	A	A	A	A	A		
	BMAC122	90	A	A	A	A	A	A	A/B	A/B	A	A	A	B	B	A	A	A	B	A	A	A	A	A		
	EBMAC871	90	A	A/B	A	A	A	A	A/B	A/B	A	A/B	A	B	B	A	A	A	B	A	A	A	A	A		
	EBMAC708	115	A	A	A	A	A	A	A	A	A	A	A	B+	B	A	A	A	A	A	A	A	A	A		
	HVM062	115	A	A/B	A	A	A	A	A	A	A	A	A	B	B	A	A	A	A/B	A	A	A	A	A		
4H	GMS089	23	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	n/r	B	A	A	A	A	A		
	BMAC310	29	A	A/B	B	B	B	B	A	A	B	B	A	A	A/B	A	A	A	B	A	A	B	B	A		
	BMAC53	30	B	A/B	B	B	B	B	A	A	B	B	A	A	A/B	B	B	B	B	A	B	B	B	A		
	BMAC884	39	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	A	A	A	A	A		
	HMLOH1A	70	A	A/B	B	A	A	A/B	A	A	A	B	A	A	A	A	A	B	A	A	A	B	A	A		
	BMAC419	86	A	B	*	A	*	A	A	A	A	*	A	A	*	A	A	B	A	A	A	B	B	A		
	BMY1	102	A	A/B	A	A	A	A	A/B	B	A	A	A	A	A	A	A	A	A/B	B	A	A	A	A/B		Bamy1
5H	BMAC113	29	A	B	B	A	A	A	A	A	A	A	A	B	B	A	A	A	B	A	A	B	B	A		
	BMAC887	29.5	A	B	B	A	A	A	A	A	A	A	A	B	B	A	A	A	B	A	A	B	B	A		
	BMAC096	33	A	B	B	A	A	A	A	A	A	A	A	B	B	A	A	A	B	A	A	B	B	A		
	GMS061	33	A	B	A	A	A	A	B	A	B	A	A	B	B	A	A	A	B	B	n/r	A	A	A		
	BMAC822	58	A	B	n/r	B	A	A	B	B	A	A	A	B	B	A	A	A	B	B	B	B	B	B	CCN Ha4	
	HVLEU	79	A	B	B	A	A	A	A	A	B	B	A	A	A	B	A	A	A/B	A	A	B	B	A		
	GMS01	105	A	A	n/r	n/r	A	A	n/r	A/B	n/r	n/r	A	B	n/r	A	A	n/r	B	A	n/r	n/r	n/r	n/r		Malt extract
6H	BMAC316	8	A	B	n/r	B	A	B	A	A	n/r	A/B	A	A	A	A	A	B	A	A	A	A	B	B		
	BMAC173	73	A	A	A	A	A	A	B	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A		
	BMAC040	145	n/r	A	A	A	A	A	A	A	A	A	A	B	B	A	A	A	B	A	A	n/r	A	A		
7H	BMAC806	16	A	B	B	A	B	B	B	A	A	B	B	A	A	A	B	A	A	A	A	B	B	A		
	BMAC167	65	B	*	A	B	B	B	B	A	B	A	B	A	A	B	B	A	A	B	B	A	A	B		
	EBMAC827	65	B	A	A	B	B	B	n/r	B	B	A	B	A	A	B	B	A	A	B	B	A	A	B		
	BMAC21	70	B	A/B	A	B	B	B	B	B	B	A	B	A	A	B	B	A	A	B	B	A	A	B		
	BMAC607	74	B	A	A	B	B	A	B	B	B	A	B	A	A	B	B	A	A	B	B	A	A	B		
	BMAC817	79	B	A	A	B	B	B	B	B	B	A	B	A	A	B	B	A	A	B	B	A	A	B		
	HVSS1	79	B	B	A	B	A/B	A/B	A	A	B	B	A/B	A/B	n/r	B	B	B	A	B	B	B	A	n/r		
	AWBMS37	80	B	A	A	B	B	B	B	B	B	A	B	A	A	B	B	A	A	B	B	A	A	B		
	HVCMA	85	B	B	A/B	B	B	B	A	A	B	B	B	B	A/B	B	B	B	A	B	B	B	A	A		
	BMAG 120	100	A	B	B	*	B	B	n/r	B	A	B	*	*	*	A	A	n/r	*	A	*	B	B	n/r	seedling sf	
	BMAC135	170	A/B	B	B	n/r	A	n/r	B	B	n/r	B	n/r	B	B+	n/r	n/r	n/r	n/r	B	B	B	A/B	A/B		
C CNR			seg	seg	seg	r	s		r	seg	r	s	s	r	r	s	s	s	r	r	r	r	r	r		
	%Gairdner		77	62	78	63	60	65	73	76	64	80	73	63	64	67	65	79	56	73	72	70	75	78	70	

Keel alleles are shown in blue (with designation of "B"), Gairdner alleles are shown in yellow ("A"), heterozygotes in green ("A/B") and 'no result' is shown in white ("n/r"). The primers are shown in column 2 together with their position on a consensus map in column 3. In the column headed 'Keel alleles' are genomic regions where MAS was applied for Keel traits and in the column headed 'Gairdner alleles' are genomic regions where MAS applied for Gairdner alleles. The resistance to CCN is shown on the bottom row (as determined by a bio-assay) and can be compared to marker alleles for locus BMAC222 on chromosome 5H.

19.9.4 Transfer of QTLs

There are now several examples of backcrossing to transfer QTLs in barley. These include quantitatively inherited Stripe rust resistance (Toojinda *et al.*, 1999), distilling quality (Powell, pers. comm.), malting quality (Thomas *et al.*, 1995; Han *et al.*, 1997; Hayes *et al.*, 1993) and boron toxicity tolerance (Jefferies *et al.*, 1999). The Australian National Barley Molecular Marker Programme has progressed with the analysis of the genetic control of malt quality traits to the point where MAS is possible for malt extract, diastatic power and wort viscosity.

In its simplest form, there is little difference between the backcross strategy required to transfer a QTL and that for a major gene. Nevertheless, there are

important differences. Firstly, there is ambiguity about the location of the QTL, often making the use of flanking markers necessary. This usually increases the size of the introgressed segment, unless the region of interest is finely mapped. Finally, the most important issue becomes validation of the QTL. This process involves testing the phenotypic effect of the particular chromosomal region derived from a mapping population, initially in a range of closely related germplasm, then in progressively less related germplasm. Collins *et al.* (1999) and Coventry *et al.* (1999) have undertaken this task for malt extract and for diastatic power, respectively, and have recommended QTLs that are amenable to marker-assisted introgression. The opportunity to manipulate complex quality traits via MAS and backcrossing is now available. This was not previously possible without time-consuming progeny testing cycles in a conventional backcrossing system using phenotypic selection. The other issue important to QTL transfer is accuracy of selection. Two studies (Lande and Thompson, 1990; Zhang and Smith, 1992) have shown that MAS significantly improved the accuracy of selection for traits of low heritability.

Example 4: Feed barley conversion — Transfer of malt quality alleles from cvs. Alexis, AC Metcalfe and Haruna nijo to the disease-resistant feed barley cv. Keel.

Typically, when breeding malt quality barley varieties, we attempt to limit the percentage of feed-type germplasm in a pedigree to a maximum of 25 percent, as the genetic control of malting quality is complex and top quality is difficult to retain if we introgress too much genetic material from inferior lines. However, resistance to five leaf diseases, plus CCN, plus adaptation to our tough Australian growing conditions, is also a complex genetic assignment. Recent advances in the genetics of malt quality and in MAS for malt quality have provided the possibility of converting feed-quality lines into malting varieties – a strategy opposite to conventional wisdom.

In our programme, progress in breeding for yield and disease resistance has been more rapid in feed barley than in malting barley, for several reasons:

- the number of breeding priorities set for malting barley (>12) compared with feed (6–7) diminishes the rate of progress;
- physical quality specifications have been much easier to achieve in feed barley; and
- a wider range of germplasm is of direct use in the improvement of feed barley than in malting barley.

Consequently, there is at least 10 percent difference in yield between malt and feed varieties, as well as significantly poorer disease resistance in the malt varieties.

A current project aims to introgress the key genetic loci influencing malt extract, diastatic power and fermentability from Canadian, European and Japanese material into elite Australian feed lines. The initial focus is on the feed variety Keel (released in 1999), the second phase will focus on WI3385 (expected for commercial release in 2003), and new recurrent parents will be added as elite lines are identified in the feed barley breeding programme.

A breeding strategy to convert elite feed varieties into malting quality lines has been developed based on transferring the important genes for malting quality from cvs. Alexis, Haruna nijo and AC Metcalfe into feed varieties. The strategy is based on backcrossing with extensive

use of molecular markers for the most rapid and efficient development of new lines. In the first year of the project, this approach has successfully developed cv. Keel backcross lines (BC_1 and BC_2) that carry the key quality genes from the three malting barley parents, including malt extract, on chromosomes 1H, 2HS, 2HL and 5H, and the ß-amylase gene on 4H, which influences diastatic power and fermentability. These lines were to enter field trials in 2002 for selection based on agronomic performance, prior to yield trials and malt quality evaluation in 2003.

The key malting quality genes from cvs. Alexis, Haruna nijo and AC Metcalfe are also being combined by merging the three cv. Keel backcrossing programmes. BC_3 generations will also be developed for each of the Keel backcrossing programmes. Molecular markers will be used to identify elite individuals from these intercrosses and the BC_3F_1 generations. The elite lines will also be used to develop doubled haploid populations by the end of 2003.

19.9.5 Simple graphical genotypes to demonstrate ancestry

Example 5: Use of graphical genotypes presented in a spreadsheet to show the contribution of the landrace barley CI 3576 to modern Australian varieties.

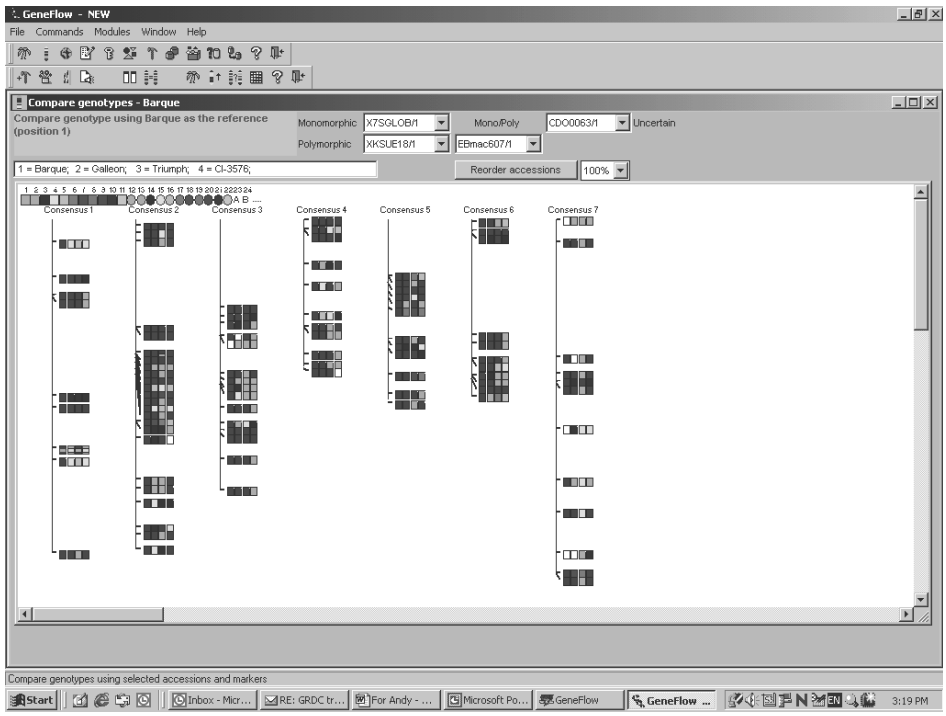
Atmojdo (2002), in an unpublished Master's thesis of the University of Adelaide, developed simple graphical genotypes of a number of Australian barley varieties of descent from an important land race known as CI 3576. The genetic composition (Figure 19.6) of the cultivars implied that something important from CI 3576 in the region around Bmac093 is retained during breeding and selection in Australia. Subsequently, mapping studies revealed that a key development locus with an allele conferring early flowering was contributed from CI 3576.

FIGURE 19.6

Graphical genotype of chromosome 2H of barley for 9 Australian barley cultivars derived from the landrace CI 3576, showing the retention of key linkage blocks associated with adaptation and disease resistance

Cultivars are shown in columns and marker loci in rows, with alleles for various SSR and RFLP loci shown both by colour (CI 3576 alleles in red) and fragment size and allelic designation. (Atmojdo, unpublished M.Ag.Sc. Thesis).

FIGURE 19.7
Graphical genotypes prepared in Geneflow® of Barque, Galleon, Triumph and CI 3576 comparing alleles at all available SSR loci



19.9.6 More sophisticated graphical genotypes to analyse flow of alleles in breeding programmes

Example 6: The use of Geneflow® software to analyse and present the contribution of parental genotypes to the breeding of the modern feed barley cv. Barque.

For whole-genome strategies to be easily interpreted and ultimately implemented in practical breeding, analysis and visualization of the data becomes crucial. In Example 5, the use of an Excel™ spreadsheet was demonstrated. A more powerful tool is Geneflow® (http://www.geneflowinc.com/). This package can manage pedigree, genotypic and phenotypic databases, and perform analyses combining this information to:

- give graphical genotypes;
- follow allele flow through complex pedigrees;
- compare genotypes from pedigree and backcross programmes against the preferred genotype; and
- much more!

One example is shown in Figure 19.7, where the contributions of parental genotypes (cvs. Triumph and Galleon – and one Galleon's key parents is CI 3576) to a new feed barley, cv. Barque, are presented across the 7 chromosomes of barley.

19.10 THE FUTURE OF MAS

There are several developments that we can expect in marker technologies over the next few years. The techniques for assaying

DNA variation between lines will become cheaper and allow higher throughput than is at present possible. While the cost per assay may come down, the cost of running an advanced molecular marker lab will go up, reflecting the need for sophisticated DNA analysis and detection systems and robotics. The cost of DNA isolation will continue to be a major limitation, although this should also come down to some extent. We can also confidently expect the number of useful traits tagged with molecular markers to increase still further. The application to crop improvement of genetic engineering, Targeting Induced Local Lesions in Genomes (TILLING), and chimeric DNA gene engineering will further increase the number of genes (loci) that will be monitored through a breeding and selection programme. These trends will encourage breeders to conduct detailed molecular analyses of lines and will greatly expand the amount of information that is available on each line. With these points in mind, we believe that the following issues will dominate the development and application of MAS.

19.10.1 Polymorphic markers

In many implementation laboratories, lack of polymorphism amongst parents is now the most important factor limiting MAS applications, especially for screening top-cross and four-way cross F_1s. Depending on the purpose of the MAS application, many more markers than are currently available will be required. For instance, for whole-genome scanning, assuming Polymorphic Information Content (PIC) = 0.25, coverage every 10 cM and a genome size of 1200 cM, 480 SSRs would be required. If MAS at targeted loci is required, the number of SSRs may in practice be around 10 to account for most parental combinations.

19.10.2 Validation of markers

Most groups undertaking MAS underestimate the cost of the validation process required after the initial identification of a potential region of interest in the mapping population. In many cases, finding a linked marker in a mapping population has proved relatively straightforward, whereas implementing it in a pragmatic breeding programme has not. The validation process is defined as the testing of an allele for its effect in genetic backgrounds other than the original mapping population, the characterization of the polymorphisms for a range of candidate markers in all combinations of parents, and the bench testing of the selected enzyme-marker/marker-protocol. Approximately 15–25 percent of the total MAS resources may be devoted to this process in some marker laboratories. The attractiveness of MAS would therefore be greatly enhanced by reducing the overheads associated with validation. It may be that diagnostic markers, such as SNPs, are one possible way to reduce these costs.

19.10.3 Marker throughput

The limitations on marker throughput were discussed briefly in the section on early-generation screening.

The primary limitations are:

- the cost and time for DNA extraction. Rapid DNA isolation systems are available that may reduce the cost but, as mentioned above, this will continue to be a major cost in MAS;
- the availability of closely linked, polymorphic markers. Public and private efforts in barley genomics may provide sufficient SSR markers to supplement those currently available and thereby overcome this limitation. Some breeders still have concerns about the use of MAS leading to narrowing of the germplasm pool to only

those parents and traits for which validated, polymorphic markers are available;
- staff resources for handling the plants, DNA and marker allele data. Many breeding programmes have funded their initial work in MAS from special funds. The time is fast approaching for public-sector breeders, and did several years ago for private breeders, where resource re-allocation within the breeding team is required to further implement MAS;
- new, robotic systems may be capable of analysing 10^4 to 10^5 loci per day, and will challenge the information processing capability of all but the largest breeding programmes; and
- the cost of robotics, Taq polymerase, non-gel-based discrimination systems, etc., for true, high-throughput systems. While such systems show great potential for handling targeted numbers, they appear at present to be beyond the budget of most barley breeders. However, SNP markers may be analysed on chips or use mass spectrophotometric methods, which could greatly reduce costs.

19.10.4 Risks and limitations of using MAS

The risk most frequently noted is the temptation to use only parents for which either markers or polymorphic markers exist, thus narrowing genetic diversity. In particular, this may concentrate use of a few, well-characterized disease-resistance genes to the exclusion of less well documented sources. This risk can be minimized by breeder discretion allocating a proportion of the programme to new or uncharacterized sources. It might be more useful to think of this problem as a challenge: How can marker technology be used to expand our useful gene pool?

There is also concern that a strong emphasis on markers in a breeding programme may lead to a focus on breeding strategies based on the technology rather than on the most appropriate strategy for a particular environment. For example, backcrossing is highly amenable to MAS, but is a conservative breeding strategy and should not become the prime focus of a breeding programme. Again, this issue can be addressed if the breeders are aware of the potential problem and include a diversity of strategies in their programme.

Another limitation is the power of QTL discrimination (Melchinger *et al.*, 1998). Many QTLs indicated in mapping population studies 'disappear' in validation populations. These QTLs may have been cross specific, subject to genotype environment interaction effects or illusory. Illusory QTLs may have been artefacts of small mapping populations, error in the phenotyping experiments or reflect fundamental limitations of QTL analysis methods.

19.10.5 Application of MAS to yield improvement

New approaches to the improvement of grain yield remain the Holy Grail of plant breeding. Yield, according to conventional wisdom, is conditioned by many genes of small effect, so how can MAS play an important role in manipulating such a complex trait? Many mapping studies have measured grain yield and applied QTL analysis (Teulat *et al.*, 2001; Marquez-Cedillo *et al.*, 2001; Baum *et al.*, 2001). Australian researchers have also measured grain yield in 10 barley mapping populations (Higgins *et al.*, 2003). The number of QTLs for grain yield varied widely, from two up to eight. This range will be due to:
- the population size of the mapping population and quality of the maps, which affects the resolution possible;

- the nature of the parents and the number of genes segregating;
- difficulties with detecting QTLs affecting yield when major genes controlling development (e.g. photoperiod response) or stature (e.g. *sdw* gene for semi-dwarfism) are segregating in the population;
- genotype × environment interaction. A number of studies report QTL × environment interactions (Piepho, 2000; Teulat *et al*., 2001; Marquez-Cedillo *et al*, 2001; von Korff *et al.,* 2008); and
- complex genetic interactions that cloud the QTL analysis.

While barley breeding programmes are successfully using MAS to transfer major genes and QTL for quality (Iguarta *et al*., 2000) and Stripe rust (Hayes, Toojinda and Vivar, 1999), progress with transferring QTLs affecting yield is less impressive. Kandemir *et al*. (2000) attempted to transfer three QTLs identified in the Steptoe × Morex mapping population into an elite malting line (cv. Morex) by marker-assisted backcrossing. The near-isogenic lines (NILs) developed did not have improved yield, although traits often related to yield, such as plant height, maturity, etc., were changed. They concluded that these yield QTLs must interact with other genes in the donor parent to give full expression, or, alternatively, they may affect harvestable yield through reduced lodging and head shattering, which may have been observed during the evaluation of the Steptoe × Morex mapping population, but not during the evaluation of the NILs.

To improve the likelihood of success, a number of steps must be taken. First, the mapping population sizes used must be increased: in the Australian barley context, population sizes range from 80 to 250, with a median of 180. The design and analysis of mapping population experiments is also important, and with appropriate techniques, such as spatial analysis and simultaneous yield and QTL analysis, it will be possible to more accurately define yield QTLs. Alternatively, different approaches, such as association mapping, may be more suitable. Clearly, it is crucial to know what other traits are coincident with the yield QTLs and it is important to seek the underlying cause of the effect of a QTL on yield. Only then will it be possible to confidently expect progress in this challenging area.

Mapping techniques may never have the precision to confidently define more than a handful of QTLs for any one trait, and this role may lie with genomics approaches.

In maize simulations, Bernardo (2001) shows that application of MAS in selection for traits controlled by very large numbers of genes might even be counterproductive. He argues that MAS has its biggest advantage over phenotypic selection when the number of genes is small (<10).

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CHAPTER 20

Coping with and exploiting genotype-by-environment interactions

Paolo Annicchiarico

20.1 INTRODUCTION

Future increase in agricultural production is limited by both the difficulty of expanding the cultivated land area (Evans, 1998), and the demands of a high-input model of agriculture (Conway, 1998). Ongoing climatic changes are expected to increase the frequency and severity of drought and other abiotic stresses in many tropical, subtropical and Mediterranean areas (Fischer, Shah and Velthuizen, 2002). To improve the availability and stability of crop production, national or regional breeding programmes need to define a strategy to produce (when economically convenient) improved germplasm capable of maximizing the agricultural potential of specific farming systems while minimizing the occurrence of crop failures or very low yields. Some major elements of this strategy are discussed in this chapter, which focuses on the difficulties and opportunities related to breeding for environments that may differ in space (across locations), in time (across cropping years) and in crop management. This section will introduce the main relevant concepts and definitions. Two following sections will be devoted to targeting of germplasm to attain optimal adoption, and breeding strategy. Two final sections will briefly discuss the control of micro-environmental variation and suitable support software.

Following on, the term 'genotype' usually designates a cultivar (genetically homogeneous, e.g. a pure line, or heterogeneous, e.g. an open-pollinated population) rather than an individual's genetic make-up. Environment pools the set of climatic, soil, biotic and management conditions for the crop in a given location-year (annuals) or location-crop cycle (perennials) combination. The target region of a breeding programme comprises the population of potential environments for the crop. It could be defined geographically (e.g. the cropping area for a national programme), or possibly by the relative frequency of major types of environment.

Purely environmental yield effects, reflecting the different ecological potentials of sites, years and management conditions, are not of direct concern for breeding or targeting varieties. Genotype main effects (i.e. differences in genotype mean yield) provide the only relevant information, when genotype × environment (G×E) interaction effects are absent or ignored. G×E effects that lead to inconsistent genotype ranking across environments are frequently too large to be ignored. They tend to be large when there is wide variation among genotypes for traits conferring tolerance to one or more stresses (e.g. drought; low or high temperatures; soil salinity; nutrient deficiency; pests; diseases; grazing) and, concurrently, wide variation among target environments for incidence of the same stress(es) (Kang, 1998). Landraces and old cultivars tend to respond relatively better in less favourable environments (Ceccarelli, 1994; van Oosterom, Bidinger and Weltzien, 2003), although the opposite response has also been observed for material that evolved in favourable environments (Annicchiarico and Piano, 2005). The level of matching of genetic determinants of phenological development (e.g. photoperiod and vernalization requirements) with site factors that affect the length of the growing season may also imply large G×E interaction across fairly vast regions (Wallace, Zobel and Yourstone, 1993). Finally, differences in genetic structure among genotypes may contribute to G×E interaction, because variety types characterized by low levels of heterogeneity (e.g. pure lines, clones, single-cross hybrids) or heterozygosity

(e.g. pure lines) are less buffered against environmental variation than other types, such as open-pollinated populations or mixtures of pure lines, owing to their lower richness in adaptive genes (Becker and Léon, 1988).

A few widely used terms, such as adaptation and yield stability, need be unequivocally defined at this stage. In an evolutionary biology context, adaptation is a process, adaptedness is the level of adaptation of plant material to a given environment, and adaptability is the ability to show good adaptedness in a wide range of environments. In a plant breeding context, the first two terms relate to a condition rather than a process, indicating the ability of the material to be high-yielding in a given environment or given conditions (to which it is adapted) (Cooper and Byth, 1996). Genotype adaptation is usually assessed on the basis of yield responses (although other variables, e.g. gross benefit, may be considered), and undergoes modification when better performing material becomes available. Breeding for wide adaptation and for high yield stability and reliability have sometimes been considered one and the same, insofar as the latter two terms indicate a consistently good yield response across environments. However, only the adaptive responses to locations, geographical areas, farming practices or other factors that can be controlled or predicted prior to sowing can be exploited by selecting and growing specifically-adapted genotypes. For example, the knowledge of specific adaptation to past years, as shown by positive genotype × year (G×Y) interaction effects, cannot be exploited in future years, as the climatic conditions that generate year-to-year environmental variation are not known in advance. Therefore, some authors have proposed applying the yield stability concept only in relation to genotype responses over time, using the adaptation concept in relation to responses in space (Barah *et al.*, 1981; Lin and Binns, 1988). This view, accepted here, agrees with the farmer's view that location is a constant—not variable—factor, and yield consistency over time is the only relevant component of a genotype's yield stability.

Breeding for wide adaptation aims to develop a variety that performs well in nearly all the target region, whereas breeding for specific adaptation aims to produce different varieties, each of which performs well in a definite area (subregion) within the region. Early plant breeders advocated the usefulness of selection for specific adaptation (Engledow, 1925), and Falconer (1952) suggested that specific breeding may be preferable for environmentally-contrasting subregions on the basis of selection theory. However, breeding programmes have mostly considered G×E interactions as simply a hindrance to crop improvement, while pursuing, even in less developed countries, a wide-adaptation strategy that tended to promote varieties with high yield potential alongside technical packages designed to significantly improve the environment (Simmonds, 1979: 356). This trend has been favoured by the perspectives of rapid yield gain offered by high input levels, the greater profitability of targeting seed markets in more productive areas, and the belief that selection in favourable areas produces a substantial yield gain also in less favourable areas. The difficulty in sustaining and expanding high-input agricultural systems, and the mounting evidence of the dimension of G×E effects demonstrated between favourable and stress-prone environments (e.g. Ceccarelli, 1989; Bänzinger, Bertrán and Lafitte, 1997), have led to reconsideration

of some opportunities offered by G×E interactions (Simmonds, 1991; Ceccarelli, 1996). In particular, G×E effects can be exploited to select and grow varieties that show positive interaction with the location and its prevailing environmental conditions (exploitation of genotype × location interaction), or low frequency of poor yield or crop failure (exploitation of within-site genotype × year interaction). Coping with G×E interactions, rather than ignoring them, is also required when breeding for wide adaptation (e.g. Cooper *et al*., 1995).

In fact, only the genotype × location (G×L) interaction that is repeatable in time can be exploited by selecting and growing specifically-adapted material. The non-repeatable G×L interaction is the genotype × location × year (G×L×Y) (or the genotype × location × crop cycle) interaction in the analysis of variance (ANOVA) of multi-site, multi-year data sets (also called Multi-environment Trials or MET), in which the time factor is crossed with location. This term and the G×Y interaction are pooled in the within-site G×Y interaction in ANOVA models holding the time factor nested into location. The term including the non-repeatable G×L interaction acts in all cases as the error term for (repeatable) G×L interaction (see Section 20.2.2). Analysing G×L effects instead of G×E effects makes the genotype adaptive responses consistent with the proposed concept of adaptation, and simpler to model (see Section 20.2.1). Several reports summarized by Annicchiarico (2002a) indicate the need for repetition in time of genotype testing in annual crops, because the estimation of G×L effects based on a single year's data tends to be largely inflated by non-repeatable effects, due mainly to within-site, year-to-year variation in climatic factors. Repetition in time may not be needed for perennials on the ground of results for alfalfa (Annicchiarico, 1992, 2002b), suggesting that the variation in environmental factors encountered by genotypes across a three-year crop cycle is wide enough to act as a buffer against the occurrence of non-repeatable G×L effects.

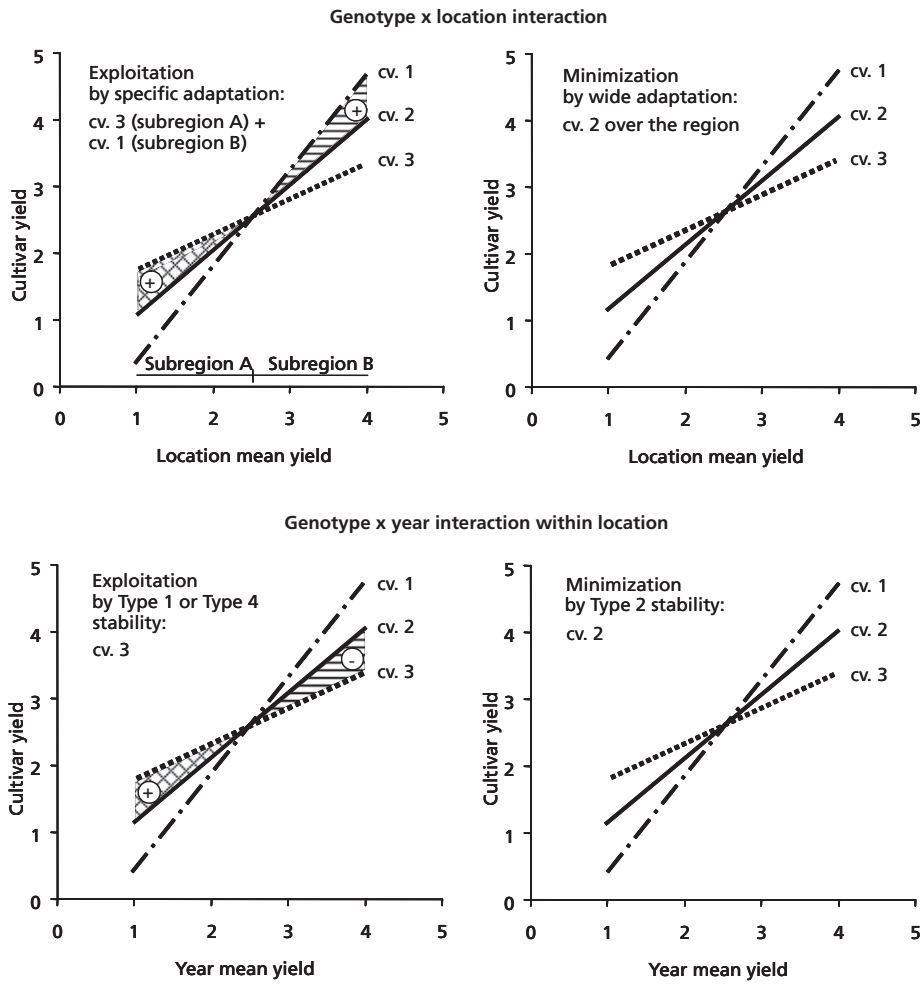
Repeatable G×L interaction effects can be exploited by breeding and growing specifically-adapted varieties, or minimized by selecting and growing widely-adapted material. The implications of either choice are shown graphically in Figure 20.1 (upper part) with respect to three hypothetical high-yielding cultivars that differ for adaptive response, as revealed by a simple model, i.e. the response to site mean yield. Minimizing G×L effects by growing the cultivar with lowest G×L interaction over all the region, i.e. cultivar 2, implies a yield penalty relative to growing specifically-adapted germplasm, i.e. cultivar 3 in the low-yielding subregion A along with cultivar 1 in the high-yielding subregion B. Likewise, aiming at selecting a variety like cultivar 2 in the context of a wide-adaptation strategy may imply lower yield gains over the region than breeding distinct germplasm for each subregion. However, the choice between exploiting or minimizing the G×L effects requires assessment of the yield gains at parity of costs (see Section 20.3.4).

The G×E interactions with the time factor can also be either exploited or minimized, by growing or selecting germplasm according to either of two concepts of stability. The first, termed 'static' by Becker and Léon (1988), is analogous to the biological concept of homeostasis: a stable genotype tends to maintain a constant yield across environments. A stable genotype according to the 'dynamic' concept of stability implies a yield response in each environment that is always parallel to the

mean response of the tested genotypes, i.e. zero G×E interaction. Lin and Binns (1988) proposed a second stability measure that refers to consistency of yield across years within location (rather than across generic environments). This measure relates to the static stability concept, but is nearer to a farmer's view of stability. The effect of the stable or dynamic concept of stability is displayed in Figure 20.1 (lower part) with respect to within-location response to year mean yield of three hypothetical cultivars. Growing cultivar 2 in all years, the most stable cultivar according to the dynamic concept, minimizes the within-site G×Y interaction effects. Growing the most stable material based on the static concept, i.e. cultivar 3, minimizes the year-to-year yield variation and implies a yield penalty in favourable, high-yielding years and a

FIGURE 20.1

Exploitation by specific adaptation, and minimization by wide adaptation, of spatial G×E interaction (above); and exploitation by the static yield stability concept, and minimization by the dynamic yield stability concept, of temporal G×E interaction (below)



yield gain in unfavourable, low-yielding years. Exploiting positive G×Y effects in unfavourable years increases the security of food production or agricultural income at national and household levels (Simmonds, 1991), making the static stability concept far more attractive than the dynamic one in a wide range of cases, and particularly for public institutions and less favoured agricultural regions.

High yield stability may be associated with low mean yield (or low stability with high mean yield), complicating variety targeting or selection. As an extreme example of high stability associated with low yield, consider a genotype that yields just above zero (high static stability) or is consistently the bottom-yielding (high dynamic stability) across years or environments. Obviously, a less stable, higher-yielding genotype would be preferable. The practical interest of combining high levels of mean yield with yield stability has led to development of the yield reliability concept. A reliable genotype has consistently high yield across years (or environments) (Section 20.2.7).

Farmers' practices frequently imply an awareness of the effects of G×E interactions. Landraces are preferred to improved varieties in stress-prone areas, reflecting their specific adaptation and higher yield stability (Almekinders, Lowaars and Bruijn, 1994). Harvest security for farmers producing near subsistence level is associated with wider crop heterogeneity, obtained by mixture and multiple cropping of different landraces (Clawson, 1985). In relatively favourable areas, mixing landrace and improved variety seed, and introgressing varietal germplasm into landraces, improves crop reliability via better response to favourable years (vom Brocke *et al*., 2003). The integration of farmers into national or regional breeding programmes fits well and enlarges the opportunities to breed for specific adaptation and yield stability (Section 20.3.1).

The adoption of biotechnologies (marker-assisted selection; genetic engineering) will hardly eliminate the need to cope with G×E interactions, because genetically based trade-offs between yield potential and tolerance to major stresses (e.g. drought; Ludlow and Muchow, 1990) and the need to choose a definite level of earliness for grain crops (Wallace, Zobel and Yourstone, 1993) will limit the possibility of assembling all useful genes in a single variety. Breeding for specific adaptation may even broaden the scope for marker-assisted selection, because a large portion of QTLs (Chapter 2 in this volume) and useful markers can be environment specific (e.g. Romagosa *et al*., 1999; Ribaut *et al*., 2007).

Targeting varieties, and defining a breeding strategy in relation to G×E interactions, are distinct objectives and will be treated separately, as they require in part different assumptions and analytical techniques, even when they can be investigated using the same set of multi-environment experiment data. Information will be given on a subset of techniques considered of primary interest on the grounds of the information generated, the ease of application (also in relation to software availability) and the limited amount of input data required. Most of them are discussed in greater detail in the book by Annicchiarico (2002a). Valuable information on general or specific aspects can also be found in the books of Gauch (1992), Basford and Tukey (2000) and Yan and Kang (2003), and in those edited by Cooper and Hammer (1996), Kang and Gauch (1996) and Kang (2002), as well as in papers cited hereafter for specific issues.

The modelling techniques described in Section 20.2 can also be used by breeding programmes for purposes other than variety targeting, e.g. for studying the adaptive responses of genotypes that represent definite ideotypes or plant types, or germplasm that differs for selection method, geographical origin or the presence of specific genes (thereby verifying different working hypotheses). The same techniques could also be applied to other contexts, e.g. for assessing the adaptation and yield stability of management practices or cropping systems (Piepho, 1998) or, more generally, for studying the interaction between two factors (one or both qualitative) (Gauch, 1992).

20.2 TARGETING CULTIVARS TO LOCATION

20.2.1 Overview

Targeting cultivars is a concern of public or private seed companies, who wish to verify the area of adaptation and the agronomic value of novel germplasm. This information, needed for proper planning of marketing and advisory schemes, is particularly useful if breeding contemplated no definite adaptation target. One genotype that is top-yielding across the target region (wide targeting), or two or more genotypes each of which is top-yielding in a distinct subregion (specific targeting), may be promoted to commercial cultivar status among several candidate entries.

Targeting cultivars is also a concern of public institutions committed to MET for defining cultivar recommendations. Different recommendation domains, i.e. subregions that are the object of specific recommendation, can be identified if locations are characterized by different top-yielding genotypes. Contemplating more than one recommended cultivar can be a sensible choice, particularly for wide subregions or genetically homogeneous variety types (pure lines, clones), as it may limit the risk of disasters arising due to the unforeseen susceptibility to a biotic or abiotic stress of the only cultivar recommended for a vast area.

Genotype targeting information can be very useful for breeding programmes also for: (i) locating elite parents for crossing, or promising populations for recurrent selection; and (ii) highlighting the success and the shortcomings of their breeding work for specific areas.

G×L interaction effects are termed as 'crossover' (alias 'qualitative') when they imply a change of genotype rank across environments, and 'quantitative' when they imply a simple variation in the extent of differences between genotypes with no change in ranking across environments. The definition of subregions as sets of locations with the same top-yielding material implies that the relevant G×L effects for targeting cultivars are the crossover type which modify the top ranks of genotypes in each environment. Subregions may be defined based on geography alone, or also according to farming practices (e.g. irrigated vs. rainfed cropping). For seed companies, subregions with limited extension or negligible advantage from specifically-adapted cultivars may be merged with larger, relatively similar subregions when the additional cost of multiplying and marketing specifically-adapted varieties is likely to outweigh the expected benefit. The recommendation by public institutions of specifically-adapted cultivars is generally convenient for maximizing regional yields and increasing the biodiversity of cultivated material.

Genotype targeting exploits the information from MET to predict yield responses in future years and, as far as possible,

TABLE 20.1
Number of subregions, and predicted and observed yield gain over the pair of most-grown cultivars, for the pair of top-yielding cultivars as predicted by different methods (observed yield data; modelled data alone or interfaced with a Geographical Information System), and comparison of models for predictive ability according to two criteria, for durum wheat in Algeria

Method [1]	No. of subregions [2]	Average gain (%)[3]		Model comparison [4]	
		Prediction [2]	Validation [5]	Sum of s_C^2 (t/ha)^2	% G×L SS / % G×L DF
Observed data	15	24.4	6.9	—	—
JR	3	12.5	10.3	0.030	5.80
AMMI	3	10.7	9.7	0.031	6.03
FR	4	10.8	11.8	0.038	3.98
AMMI + GIS	2	9.4	8.6	—	—
FR + GIS	3	11.4	9.4	—	—

NOTES: (1) Key to methods: JR = joint regression, AMMI = Additive Main effects and Multiplicative Interaction with one PC axis, and FR = two-covariate factorial regression modelling; GIS = Geographical Information System. (2) Based on modelling of 24 genotypes across 17 test sites in 1998/1999 and 1999/2000. (3) Across values of 16 individual test sites; source: Annicchiarico, Bellah and Chiari, 2006. (4) See Section 20.2.6 for explanation of criteria; source: Annicchiarico *et al*., 2002. (5) In 2000/2001.

at new sites. Modelling entry yields can clarify the adaptive responses, facilitate the variety targeting, and improve the prediction of future responses. This is showed in Table 20.1 with reference to the site-specific recommendation of two top-yielding cultivars based on cultivar yields on the site as observed (entry means across two years and four replications per year) and as modelled by each of three techniques described later (neglecting here modelling interfaced with a Geographical Information System – GIS).

In comparison with observed data, modelled data implied much fewer subregions (3 or 4 instead of 15, for 17 test sites), thereby facilitating the variety targeting, and provided 3–5 percent higher yields on average in a validation data set. Modelling was a highly cost-efficient activity here: the best model allowed for nearly doubling (+71 percent) the gain from adopting better cultivars in comparison with observed data, while requiring just a modest additional cost in comparison with the evaluation costs. In addition, observed data largely overestimated the predicted gain from improved choice of cultivars, while modelled data provided realistic predictions of these gains in the validation data set (Table 20.1). Modelled data can predict cultivar differences on the site better than observed data because all model parameters (hence, all plot values used for estimating these parameters) concur to estimate each genotype by location cell mean instead of only the plot values for the specific cell mean, thereby reducing the amount of uncontrolled error variation (so-called 'noise') in the estimated G×L effect (Gauch, 1992). For trials repeated over time, the noise relates mainly to the error term for G×L interaction (i.e. G×L×Y interaction, or within-site G×Y interaction). Modelling can decrease the number of subregions because noise effects allowing lower-yielding material to occasionally appear top-yielding have been reduced (Gauch and Zobel, 1997). Modelling can also facilitate the extension of results to new sites (see Section 20.2.6).

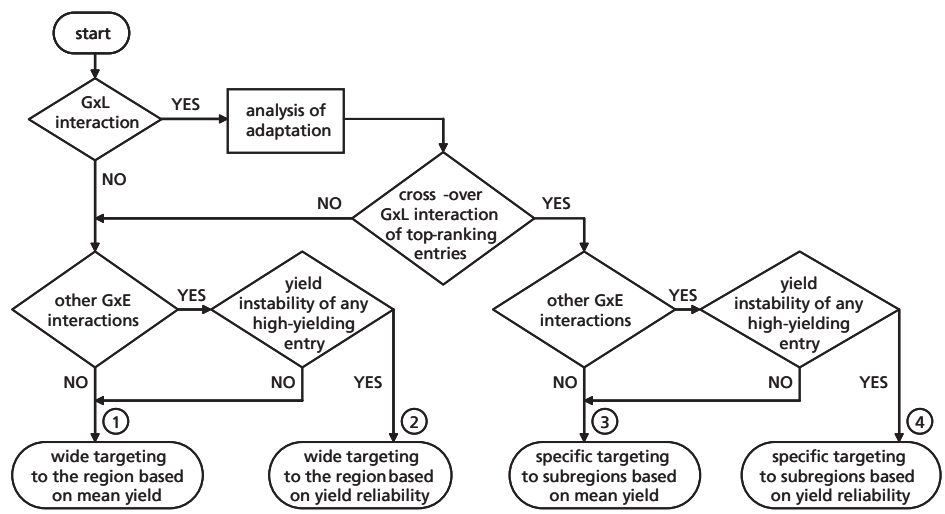
As anticipated, analysing G×L effects instead of G×E effects simplifies the modelling of adaptive responses, besides being conceptually sound. This is shown in Table 20.2 for a model described in Section

TABLE 20.2
Complexity of adaptation patterns as depicted by the number of significant principal component (PC) axes, in the analysis of genotype × environment (G×E) and genotype × location (G×L) interaction effects in six data sets

Data set	No. of sites	No. of years	No. of genotypes	Significant PC axes (1)	
				G×E	G×L
Bread wheat	31	3	18	5	2
Durum wheat 1	6	3	9	4	2
Durum wheat 2	5	2	15	4	1
Durum wheat 3	6	2	12	4	1
Maize 1	11	3	13	4	0
Maize 2	11	3	11	3	1

NOTE: (1) $P < 0.01$ according to F_{GH2} test, in an Additive Main effects and Multiplicative Interaction (AMMI) analysis.
Source: Annicchiarico (1997).

FIGURE 20.2
Flow chart of steps for targeting genotypes from analysis of multi-location yield trials repeated over time



NOTES: G×L = genotype × location; G×E = genotype × environment (environment as location–year or location–crop cycle combination).

20.2.4. Responses that are remarkably complex when evaluated on a G×E basis (requiring three or more dimensions for a convenient multivariate representation) become relatively simple on a G×L basis (requiring two dimensions at most). A simple model also facilitates the extension of results to new sites (Section 20.2.6).

The main analytical steps for targeting cultivars on the basis of multi-location yield trials repeated over time are summarized in Figure 20.2. There are four possible conclusions, which imply the targeting of same material over the region or distinct material to subregions and, in both cases, the inclusion or exclusion of yield stability

in the assessment of genotype merit. Specific targeting can be envisaged when, in the presence of significant G×L interaction in the ANOVA, the G×L effects as modelled in the analysis of adaptation imply the rank inversion of top-yielding genotypes between locations. Highly significant G×E interaction (e.g. $P < 0.001$) for ANOVA effects other than G×L interaction supports the interest of yield stability and the variety targeting based on yield reliability, but only in the presence of differences in stability among well-performing entries. Obviously, yield stability is not an issue for trials not repeated over time.

20.2.2 Types of data and ANOVA models

Genotype evaluation trials may be carried out either at research stations and experimental farms, or on farm or village land. In the former case, it is imperative that the crop management is as close as possible to that of the target population of farmers' environments (Ceccarelli, 1994). In the latter, the trials tend to have few or no replications, to reduce the number of plots per farmer site. Unreplicated trials performed contemporaneously by various farmers at nearly the same site may act as complete blocks (or large incomplete blocks) for the site, targeting two complete replications per site. Should only one unreplicated trial be adopted on each site, experimental errors may be estimated from the variation of some replicated entries randomly assigned to plots. The sample of test sites should encompass the major cropping areas and farming practices in the target region, to reflect the variation in climatic, soil, biotic and crop management factors. The number of sites may vary depending on the size of the region and the variation in environmental factors, but it should probably not fall below 5 or 6. Repetition in time is recommended for annual crops.

An inventory of useful ANOVA models with indication of relevant error terms for F tests (which depend on assumptions on genotype and location as fixed or random factors) can be found in Annicchiarico (2002a: 23). Year is always a random factor, while genotype is always fixed in the context of this section. The following class of models can be applied to trials not repeated over time (as it is frequently the case for perennials) laid out in a randomized complete block (RCB) design:

$$R_{ijr} = m + G_i + L_j + B_r(L_j) + GL_{ij} + e_{ijr}$$

where (here and in following formulae): R_{ijr} = response of the genotype i in the location j and block r; m = grand mean; G = genotype, L = location, and B = block main effects; and e_{ijr} = random experimental error. With respect to mean values of genotype (m_i) and location (m_j) and the observed genotype-location cell mean (m_{ij}), ANOVA main effects and G×L effects are estimated as:

$G_i = m_i - m$;

$L_j = m_j - m$;

$GL_{ij} = m_{ij} - m - G_i - L_j = m_{ij} - m_i - m_j + m$.

The error term for the mean square (MS) of G×L interaction is the pooled experimental error.

The following class of ANOVA models can be used for multi-location trials repeated at each site during same cropping years using a RCB design:

$$R_{ijkr} = m + G_i + L_j + Y_k + B_r(L_j\,Y_k) + GL_{ij} + GY_{ik} + LY_{jk} + GLY_{ijk} + e_{ijkr}$$

where R_{ijkr} = yield response of the genotype i in the location j, year k and block r, and Y = year (or crop cycle, for perennials)

effect. The error terms for G×E effects are: (i) G×L×Y interaction, for G×L interaction; (ii) pooled experimental error, for G×L×Y interaction; and (iii) pooled error (if location is fixed factor), for G×Y interaction.

This last class of models is particularly useful when test sites differ for number and/or timing of test years. It contemplates the year factor nested into (instead of crossed with) location:

$$R_{ijkr} = m + G_i + L_j + Y_k\,(L_j) + B_r\,(L_j\,Y_k) + GL_{ij} + GY_{ik}\,(L_j) + e_{ijkr}\,.$$

Non-repeatable G×L effects are here included in the G×Y interaction within site, which is tested on the pooled experimental error and acts as the error term for G×L interaction.

The possible heterogeneity of experimental errors is a major concern only for trials not repeated over time (where the pooled error acts as error term for G×L interaction). In this case, a transformation of variable may be envisaged when experimental errors vary as a function of the environment mean yield (Dagnelie, 1975: 367; Annicchiarico, 2002a: 28). Analysing balanced data sets is preferable (estimating missing plot values according to the design). The unbalance due to adoption of different experiment designs or to varying number of experiment replications can be overcome by performing the ANOVA on genotype–location–year cell means, estimating the pooled experimental error and converting the ANOVA sum of squares (SS) into values relative to plot data analysis, as described in Annicchiarico (2002a: 21). The absence of some genotype–location–year cell mean, or the variable number of test years per site, can be taken into account by using corrected SS (usually Type III) in the combined ANOVA, and estimating the genotype by location cell means subjected to analysis of adaptation as least squares means (i.e. adjusting for the lack of orthogonality in the data; Patterson, 1997). No observation for some genotype–location cell mean is a serious problem that may be dealt with by specific techniques and support software (e.g. Gauch, 1992: 157; 2007). A complete matrix of genotype by location cell means may also be obtained by eliminating from the data set some genotypes or locations with missing values.

Climatic, soil, biotic and crop management data of locations and morphophysiological traits of genotypes (even limited to a subset of test sites) can help identify environmental factors and adaptive traits that contribute to G×L interaction. Environmental data may also help extend the results to new sites, and are needed (from all environments) for factorial regression modelling.

20.2.3 Joint regression model

This model was developed by Yates and Cochran (1938) and proposed again, in slightly different forms, by Finlay and Wilkinson (1963), Eberhart and Russell (1966) and Perkins and Jinks (1968). In Perkins and Jinks' model (here applied to G×L rather than G×E interaction analysis), the GL_{ij} effects are modelled as a function of the location mean value (m_j) or the location main effect (L_j), which represents an indicator of the ecological potential of the site for the crop:

$$GL_{ij} = \beta_i\,L_j + d_{ij} = \alpha_i + \beta_i\,m_j + d_{ij}$$

where β_i is the regression coefficient of the genotype i and d_{ij} is the deviation from the model; in the second expression an intercept value α_i (equal to $-m\,\beta_i$) is also present. The β coefficients, with a mean value equal to zero, and the genotype mean

yields are the relevant estimated parameters of genotype adaptation. The expected (or modelled) yield response of the genotype *i* at the site *j* is:

$$R_{ij} = m + G_i + L_j + \alpha_i + \beta_i m_j = m_i + L_j + \alpha_i + \beta_i m_j .$$

Finlay and Wilkinson (1963) proposed a simpler description of genotype response to site mean yield, based on the coefficient b_i, which is equal to $(\beta_i + 1)$:

$$R_{ij} = a_i + b_i m_j$$

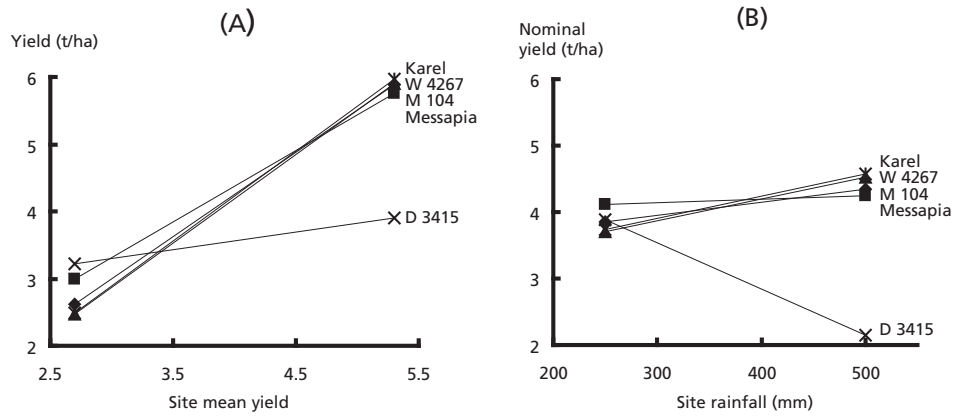
where the intercept values a_i (different from previous α_i) are equal to $(m_i - m\, b_i)$. Specific adaptation to high-yielding and low-yielding sites descends from b_i distinctly higher and lower than unity, respectively, in the presence of relatively high m_i. Conversely, b_i around unity indicates a lack of specific adaptation (and wide adaptation, if combined with high m_i). No definite indications on genotype adaptation can be inferred solely from *b* values.

The ANOVA G×L interaction SS and degrees of freedom (DF) are partitioned into two components: (i) the heterogeneity of genotype regressions, with DF = $(g - 1)$ for *g* genotypes; and (ii) the deviations from regressions, with the residual G×L interaction DF. The SS proportion accounted for by the former term, i.e. the model R^2, may be obtained: (i) by summing up the model SS across separate regression analyses of G×L effects as a function of site mean yield performed for each genotype, and calculating its proportion on the total SS of the regressions; or (ii) as the ratio of model to total SS in the analysis of covariance of all G×L effects as a function of genotype main effect and genotype × site mean yield interaction. For integration with ANOVA results (relative to plot data), the SS for the two terms can be calculated by multiplying the model R^2 and its complement to one, respectively, by the ANOVA G×L interaction SS. The genotype regressions MS should preferably be tested using the deviations from regressions MS as the error, whereas this latter MS is tested on the appropriate error MS for the ANOVA G×L interaction. It is preferable to test each *b* coefficient for difference to unity (or each *ß* coefficient for difference to zero) using the deviation from regression of the individual genotype as the error term (as provided by a separate regression analysis for each genotype). Also non-significant regression parameters should be used for modelling. The inference on expected yields is valid in the range of observed site mean yields.

Targeted genotypes depend on the site mean yield. For example, the adaptive responses of top-yielding material reported in Figure 20.3(A) suggest the presence of three subregions: (i) high-yielding (site mean yield >4.5 t/ha), where 'Karel' is top-ranking but 'W 4267' and 'M 104' yield almost as well; (ii) medium-yielding (mean yield 3–4.5 t/ha), where 'Messapia' is the top-ranking cultivar; and (iii) low-yielding (mean yield <3 t/ha), where 'D 3415' is preferable.

For this and following models, a simple outline of material statistically inferior to the top-yielding entry at variable levels of site mean yield may be provided by the mean value of Dunnett's one-tailed critical difference (Annicchiarico, 2002a: 34). The assessment is improved by computing Dunnett's critical difference for each test site on the ground of its specific error term, i.e. the G×Y interaction (for trials repeated over time) or the pooled experimental error (Annicchiarico, Bellah and Chiari, 2006).

FIGURE 20.3
Yields modelled by joint regression (A), and nominal yields modelled by factorial regression on rainfall from January to June (B), for durum wheat varieties across Italian locations



Source: Annicchiarico, 2002a.

More precise and complex methods for cultivar comparison at specific values of site mean yield (or other environmental covariate) are available (e.g. Piepho, Denis and van Eeuwijk, 1998). It is recommended to adopt less critical Type 1 error rates, e.g. $P < 0.20$, to achieve a better balance with Type 2 error rates. Even so, the site-specific recommendation of fairly large sets of statistically ($P < 0.20$) not different cultivars provided markedly lower yields than that of two top-yielding cultivars (regardless of statistical comparisons) for durum wheat in Algeria (Annicchiarico, Bellah and Chiari, 2006), confirming the high Type 2 errors (mainly due to large within-site G×Y interaction) implied by statistical differences. Type 2 errors may be very high also when the pooled experimental error acts as the error term (Kang, 1998).

20.2.4 AMMI models

The use in agricultural research of Additive Main effects and Multiplicative Interaction (AMMI) models was proposed by Kempton (1984), but became popular after Gauch's (1992) comprehensive monograph. Genotype and location main effects are estimated by ANOVA. The G×L interaction matrix is subjected to a double-centred principal components analysis (i.e. two simultaneous analyses: in the one, the genotypes are individuals and the sites original variables; in the other, vice versa), which models the G×L effects according to a multiplicative term whose estimated parameters relate to statistically significant principal components (PC) axes:

$$GL_{ij} = \sum u_{in}\, v_{jn}\, l_n + d_{ij} = \sum (u_{in} \sqrt{l_n})$$
$$(v_{jn} \sqrt{l_n}) + d_{ij} = \sum u_{in}' v_{jn}' + d_{ij}$$

where u_{in} and v_{jn} are eigenvectors (scaled as unit vectors, i.e. $\sum u_i^2 = \sum v_j^2 = 1$) of the genotype i and the location j, respectively, and l_n is the singular value (i.e. the square root of the latent root or eigenvalue), for the PC axis n; and d_{ij} is the deviation from the model. The further scaling of eigenvectors through multiplication by $\sqrt{l_n}$

allows for a straightforward estimation of the GL_{ij} effects expected on the PC axis n by multiplying the scaled genotype (u_{in}') and location (v_{jn}') scores on that axis (Gauch, 1992: 85). A genotype-location pair has a largely positive G×L effect expected on a given PC axis if the genotype and location PC scores are high and with same sign (while different signs implies a largely negative G×L effect). The simultaneous sign change of all genotype and location scores on a given PC axis leaves the estimated G×L effects unchanged.

There are several possible AMMI models characterized by a number of PC axes ranging, for g genotypes and l locations, from zero (AMMI-0, i.e. additive model) to a minimum between (g – 1) and (l – 1). The full model (AMMI-F), with the highest number of PC axes, provides a perfect fit between expected and observed data. Models including one (AMMI-1) or two (AMMI-2) PC axes are frequently appropriate in the presence of significant G×L interaction (Table 20.2). For AMMI-2 models, the scaled scores of genotypes and locations in the space of PC 1 and PC 2 may be reported in a single graph (biplot) to appreciate site or genotype similarity for G×L effects, and graphically estimate these effects. The AMMI-1 biplot displays mean values on the abscissa and PC 1 scores on the ordinate axis of genotypes and locations, showing all determinants of genotype performance.

For integration with ANOVA results, the ANOVA G×L interaction SS is divided into portions relating to each significant PC axis and to a residual term. The SS for each PC can be obtained as the proportion of G×L interaction SS accounted for by the PC multiplied by the ANOVA G×L interaction SS. The DF for the PC axis n is (Gauch, 1992: 85): ($g + l - 1 - 2n$). The G×L interaction SS and DF not accounted for by significant PC axes are pooled in the residual G×L interaction term. The F_R test is commendable for statistical testing of PC axes in a wide range of situations (Piepho, 1995; Annicchiarico, 2002a: 38). This simple test (usually not provided by statistical software) verifies the significance of the residual G×L interaction in each AMMI model, beginning with AMMI-0. By an ordinary F ratio, the MS of the residual is tested on the error MS for the ANOVA G×L interaction (for AMMI-0, the test coincides with the ANOVA F test). A significant result implies the addition of one more PC to the model (i.e. the significance of the newly-added PC).

In the selected AMMI model, the expected response of the genotype i in the location j is:

$$R_{ij} = m + G_i + L_j + \sum (u_{in}' v_{jn}') = m_i + L_j + \sum (u_{in}' v_{jn}').$$

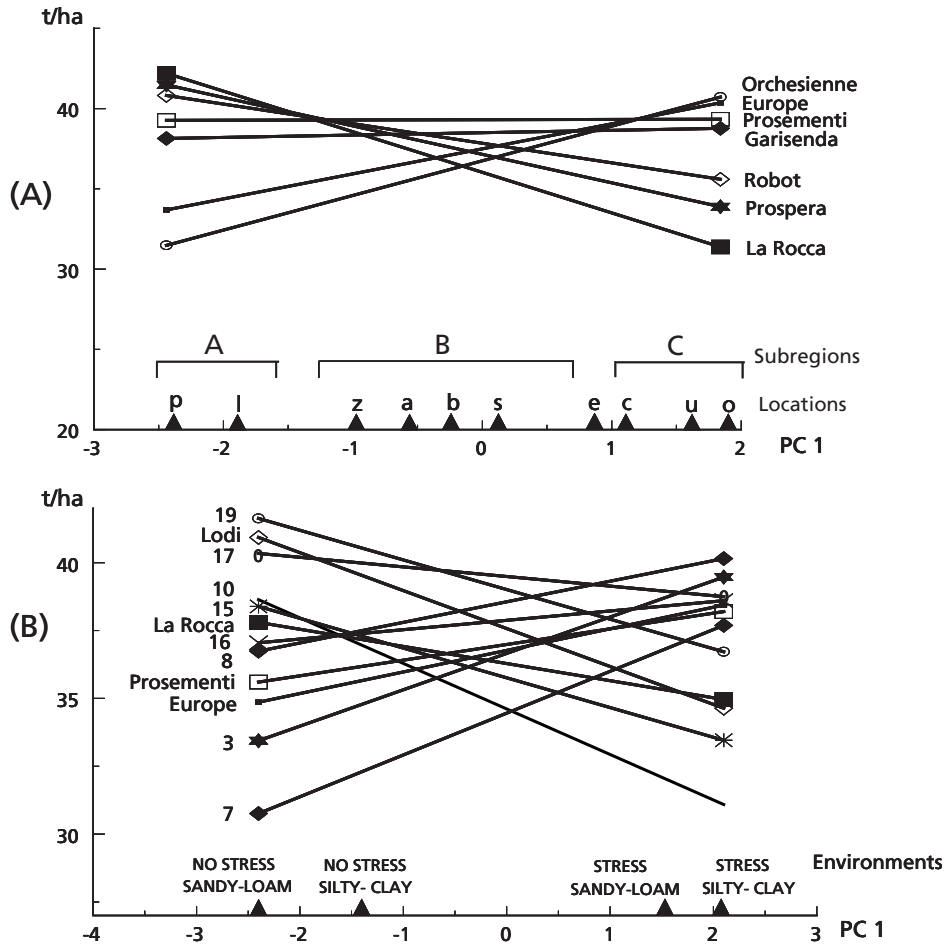
As the changes in genotype rank across sites only depend on the multiplicative term, the adaptive responses can conveniently be represented as a function of the scaled scores of locations on the statistically significant PC axes. The location main effect, which has no influence on genotype ranks and complicates the graphic representation of adaptation patterns, may be eliminated, thereby modelling the yield responses as nominal yields (Gauch and Zobel, 1997). For AMMI-1 models, nominal yields (N_{ij}) can be estimated as:

$$N_{ij} = m_i + (u_{i1}' v_{j1}').$$

They can be represented by straight lines as a function of the scaled PC 1 score of sites reported in abscissa, as shown in Figure 20.4(A) for alfalfa varieties grown

FIGURE 20.4

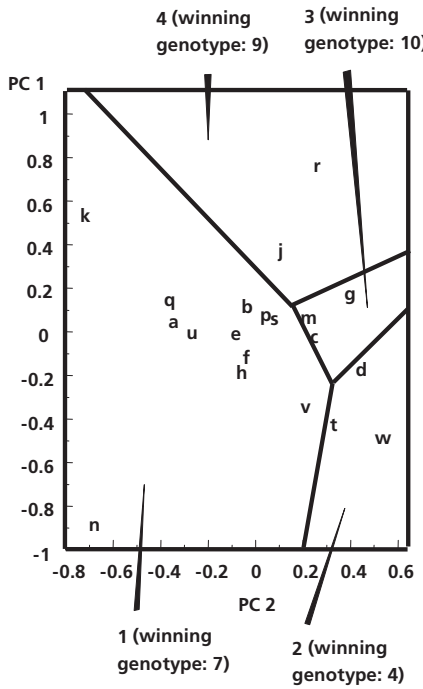
Nominal yields of top-yielding alfalfa cultivars as a function of the site score on the first G×L interaction principal component (PC) axis in a reference data set (A) or the score on the first G×E interaction PC axis of four artificial environments created by the factorial combination of two drought stress levels (almost nil or high) by two soil types (B)



Source: Annicchiarico and Piano, 2005.

in locations of northern Italy. For each genotype, N_{ij} values may be calculated just for the two sites with extreme PC 1 scores (sites 'p' and 'o'; if the software outputs R_{ij} values, $N_{ij} = R_{ij} - L_j$) and the two values connected by a straight line. The results suggest the presence of three recommendation domains: (i) 'La Rocca', 'Prospera' and (to a lesser extent) 'Robot' for the sites 'p' and 'l'; (ii) 'Prosementi' and (to a lesser extent) 'Garisenda' and 'Robot' for the sites 'z', 'a', 'b', 's' and 'e'; and (iii) 'Orchesienne', 'Europe' and (to a lesser extent) 'Prosementi' for the sites 'u', 'o' and 'c'. These subregions are almost coincident with those indicated in Figure 20.4(A),

FIGURE 20.5
Scores on the first two G×L interaction PC axes of 20 Italian locations (coded by letters), and definition of four subregions for bread wheat variety recommendation by grouping sites that have same expected top-yielding genotype (coded by number)



Source: Annicchiarico and Perenzin (unpublished results).

which actually are candidate subregions for breeding as identified by a cluster analysis of site PC scores (see Section 20.3.3).

For AMMI-2 models, the graphical expression of nominal yield responses would require three dimensions (one for each PC axis, and one for yield). However, subregions may graphically be represented as in Figure 20.5, in which the sites (displayed in the space of PC 1 and PC 2) that have same top-yielding material according to AMMI-2 modelled yields are grouped together (Gauch, 1992). This graph can also be outputted by freely-available software (see Section 20.5). In the example, genotype 7 may be targeted to 12 sites, whereas genotypes 2, 3 and 4 are of specific interest for small subsets of sites. Targeting has to be based on listed expected yields of genotypes in each test site when displaying more than one top-yielding cultivar per site in AMMI-2 models or when adopting AMMI-3 or more complex models.

Environmental variables can be related to PC scores of locations by correlation or regression analysis to reveal factors that are likely to affect G×L interaction, characterize subregions, and to scale up results (see Section 20.2.6). Likewise, correlations of morphophysiological traits (possibly recorded in a subset of sites) with adaptation parameters of genotypes (mean yield and PC scores) may reveal traits associated with specific or wide adaptation.

Other AMMI models including PC axes with different quantitative weights (instead of truncated models including or excluding a given PC axis) have been proposed to increase the predictive accuracy (Cornelius and Crossa, 1999). The genotype main effect (G) plus genotype × environment (GE) interaction (GGE) biplot analysis (Yan and Kang, 2003) is another model usable for GL interaction analysis. It applies singular value decomposition to a matrix of genotype–location cell means with the environmental effects removed (rather than a matrix of G×L effects). The first PC axis tends to summarize the genotype main effects and the other PC axes the G×L effects, but additional procedures may be needed to clearly separate these effects, while having some disadvantage relative to AMMI for graphical representations and in other respects (Gauch, Piepho and Annicchiarico, 2008).

20.2.5 FACTORIAL REGRESSION MODELS

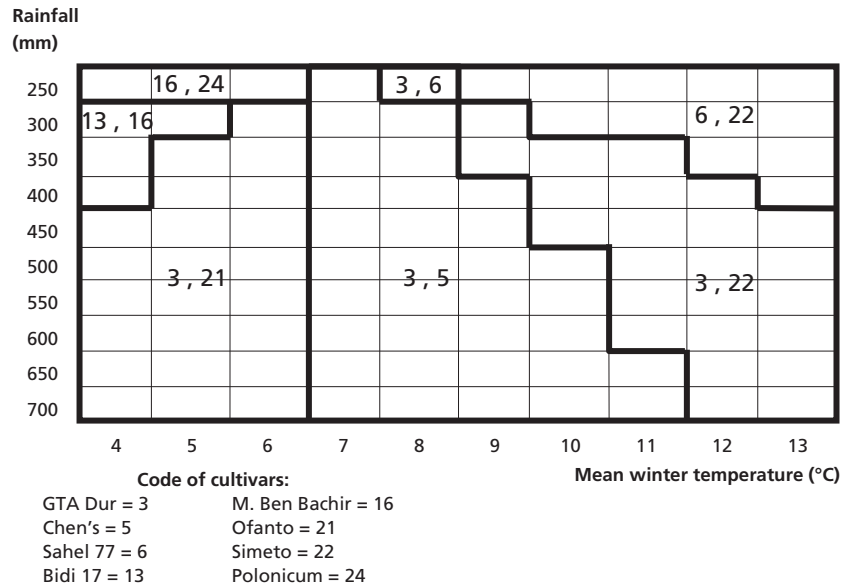
Modelling genotype adaptive responses to environmental covariates was proposed by Hardwick and Wood (1972). Covariates are usually quantitative, but qualitative ones can be incorporated through a set of dummy variables (Piepho, Denis and van Eeuwijk, 1998). Regressions are usually linear, but quadratic terms may be included as additional covariates. Denis (1988) described also the use of genotypic covariates together with environmental ones (not considered herein). The GL_{ij} effects are modelled as a function of the mean value on the site j of the environmental variable n (V_{jn}):

$$GL_{ij} = \alpha_i + \sum \beta_{in} V_{jn} + d_{ij}$$

where: α_i = intercept value, and β_{in} = regression coefficient on the covariate n, for the genotype i; and d_{ij} = deviation from the model. For one covariate equal to site mean yield, the model coincides with Perkins and Jinks' joint linear regression. Positive and negative β values indicate a positive G×L effect in sites with high and low level, respectively, of the environmental variable concerned (while specific adaptation also requires relatively high entry mean yield, besides the positive G×L effect).

The model is constructed with the progressive addition of the most important covariates (Denis, 1988). The best one-covariate model is identified on the basis of the proportion of G×L interaction SS accounted for (i.e. the model R^2), which may be computed for each covariate either: (i) by summing up the model SS across separate regression analyses of G×L effects as a function of site mean value of the covariate performed for each genotype, and calculating its proportion on the total SS of the regressions; or (ii) as the ratio of model to total SS in the analysis of covariance of all G×L effects as a function of the genotype factor and its interaction with site value of the covariate. For integration with ANOVA results, the SS for the covariate and the residual G×L interaction can be calculated by multiplying the model R^2 and its complement to one by the ANOVA G×L interaction SS. For g genotypes, each covariate accounts for $(g-1)$ GL interaction DF while the residual G×L interaction pools the remaining DF. MS values for the covariate and the residual G×L can be tested on the error MS for the ANOVA G×L interaction. After identifying the best one-covariate model, the best two-covariate model is found by comparing (on the basis of their R^2) the possible multiple linear regression models including the best single covariate (previously identified). Again, the R^2 for each two-covariate model may obtained either by summing the results of multiple regression analyses of G×L effects executed for individual genotypes, or through an analysis of covariance of all G×L effects including, beside the genotype factor, a genotype × covariate site mean value interaction term for each of the two covariates. The MS of any added covariate is the ratio between the portion of G×L interaction SS accounted for by the additional covariate, i.e. the partial regression SS (calculated as the difference in R^2 between the two-covariate and the one-covariate model, multiplied by the ANOVA G×L interaction SS), and its DF. If the added covariate of the two-covariate model with highest R^2 is significant, the best three-covariate model can be searched for, and so forth until no significant covariate can be added. The estimation and testing for difference to zero of the β_{in} parameters is limited to the selected model (preferably

FIGURE 20.6
Expected pair of top-yielding durum wheat cultivars for each of 10 annual rainfall by 10 mean winter temperature values of Algerian locations in a factorial regression model



Source: Annicchiarico *et al*., 2002.

using as error term the deviations from the model of the genotype *i*). Also non-significant estimated parameters should be used for modelling. The inference is valid in the range of observed covariate values.

Careful selection of environmental variables prior to analysis, on the basis of common sense and their putative importance in G×L interaction, is recommended, to limit the calculation process and avoid the risk of multicollinearity. This risk tends to be small (Vargas *et al.*, 1999) but may be eliminated through a partial least squares regression model (Aastveit and Martens, 1986).

The expected yield of the genotype *i* in the location *j* according to the selected model is:

$$R_{ij} = m + G_i + L_j + \alpha_i + \sum \beta_{in} V_{jn} = m_i + L_j + \alpha_i + \sum \beta_{in} V_{jn} .$$

Its expression as nominal yield:

$$N_{ij} = m_i + \alpha_i + \sum \beta_{in} V_{jn}$$

may simplify its calculation and, for one-covariate models, allows modelling of the genotype responses to the environmental variable as straight lines (connecting the N_{ij} values calculated only for the extreme covariate levels in the graph), as shown in Figure 20.3(B) for response to site mean rainfall of the same cultivars already modelled by joint regression (modelling actual yields as straight lines would require a perfectly linear response to rainfall for site mean yield). Specific targeting may be envisaged here for two subregions: (i) relatively low rainfall (<400 mm), where 'Messapia' is preferable; and (ii) relatively high rainfall (≥400 mm), where 'Karel' and 'W 4267' are preferable.

For two-covariate models, nominal yield responses would require a three-dimensional graphical representation. However, it is possible to display the expected top-yielding material and the subregions as a function of sets of site values for the covariates, as shown in Figure 20.6 for two top-yielding cultivars in each combination of 10 rainfall by 10 winter temperature levels (a denser grid of points would provide more fine-tuned indications). Targeting has to rely on lists of expected genotype yields for more complex models.

20.2.6 MODEL COMPARISON AND SCALING UP OF RESULTS

Joint regression is a simple and popular model, but cannot describe G×L effects that are ecologically complex (e.g. because of variably occurring environmental stresses) or mainly affected by a different environmental factor relative to site mean yield (Annicchiarico, 1997). Factorial regression allows for explicitly assessing the relationships of environmental variables with G×L effects, thereby improving our understanding of G×L interaction (both in general and for single genotypes). It also simplifies the definition of subregions, because the genotype responses to new sites can easily be predicted as a function of the site mean value for the significant covariates. Its use may be limited, however, by the unavailability of environmental data in the complete set of test environments, or by the modest explicative value of the available covariates. With AMMI analysis, sites with missing environmental data can be used for modelling but excluded from analyses that assess the relationships of these data with G×L effects. AMMI modelling has a broader range of application, but makes the definition of subregions less straightforward than for regression models.

The model predictive ability depends on the accuracy (high SS) and the parsimony (low DF) of its G×L interaction parameters (Gauch, 1992: 134). Uni-dimensional models (joint linear regression, AMMI-1 and one-covariate factorial regression models) can be compared for predictive ability based on the MS value of their G×L interaction parameter (which takes account of both characteristics). The sum of the estimated variances of the G×L interaction terms of the model could provide a general criterion for model comparison (Annicchiarico, 2002a: 49). The variances of G×L interaction PC axes (which are uncorrelated), or those of environmental covariates (which relate to partial regression SS for each added covariate), can be summed up because they add independent pieces of information. Extending Becker's (1984) procedure for estimating the variance of genotype regressions to PC axes or environmental covariates, the variance of any component of the G×L interaction (s_C^2) can be estimated from its MS (M_C) and the error MS for the ANOVA G×L interaction as it follows (with respect to notations in Section 20.2.2, and M_e = pooled error MS): $s_C^2 = (M_C - M_e) / r\,l$, for trials not repeated over time; $s_C^2 = (M_C - M_{GLY}) / r\,y\,l$, for location and year crossed factors; and $s_C^2 = (M_C - M_{GY(L)} / r\,y\,l$, for the year factor nested into location. Brancourt-Hulmel, Biarnès-Dumoulin and Denis (1997) proposed a second criterion equal to the following ratio of SS to DF globally accounted for by the G×L interaction parameters of the model: % G×L interaction SS / % G×L interaction DF. An empirical assessment suggested the superiority of the former criterion, whose indication of the greater predictive ability of the two-covariate factorial regression over joint regression or AMMI-1 was confirmed by the greater

ability of this model to predict the top-yielding material (i.e. the information of practical interest) in a validation data set (Table 20.1). In contrast, the Brancourt-Hulmel, Biarnès-Dumoulin and Denis' (1997) criterion ranked factorial regression as the least predictive.

The definition of subregions, initially limited to test sites, should ideally be scaled up to the entire target region. The possible procedures vary depending on the modelling technique and the availability and the type of environmental data. Whenever possible, the model established in relation to genotype responses to test year values of relevant environmental variables (site mean yield; climatic covariates; etc.) is exploited to predict future responses on the basis of long-term or mean values of these variables on new sites or test sites. Test sites may be reassigned to other subregions, if top-ranking material happens to differ for long-term conditions. One site may belong to more subregions depending on the crop management (e.g. irrigation level), if relevant. For joint regression, sites can be assigned to subregions depending on their long-term mean yield (as known from statistical records or, possibly, as predicted by mean values of relevant environmental variables). For factorial regression, nominal yields of genotypes can be estimated as a function of site mean values of the significant covariates. For models with one or two covariates, graphical expressions such as Figure 20.3(B) or Figure 20.6 can greatly simplify the scaling up (reading the best-yielding material as a function of the covariate value(s) on the site).

Extending results is more complex for AMMI models. If no environmental data is available or no relationship is found between environmental variation and ordination on significant G×L interaction PC axes of sites, subregion definition relies on the supposedly close relationship between geographical proximity and similarity for top-yielding material of the sites (attributing new sites to the subregion to which the nearest test site belongs). If a test site is representative of a given area, this area can be attributed to the subregion including the test site. Correlations of environmental variables with PC axes of sites (possibly assessed for a subset of test sites) can help characterize subregions and assign new sites to the most similar subregion according to the mean level of these variables on the site. Taking a step further, an equation for estimating the scaled PC scores of sites as a function of environmental variables recorded in test years may be searched for by a stepwise multiple regression analysis for each significant PC axis. The selected equation(s), if able to explain a fairly large portion of variation (e.g. $R^2 \geq 60$ percent), can be exploited to predict the site PC score and, thereby, the expected nominal yields of genotypes on the site (Annicchiarico, 2002a: 57).

The opportunity to interface GIS data allows for a very fine-tuned geographical definition of subregions for scaling up factorial regression or AMMI results (Annicchiarico, Bellah and Chiari, 2006). Alternatively, a simple 'Decision-aid System' could be developed that outputs the expected nominal yields of the cultivars (possibly together with an average value of Dunnett's one-tailed critical difference) as a function of the inputted site mean value of relevant environmental variables.

Scaling up may introduce a bias due to neglecting some important environmental variable or inaccurately estimating its effects. This bias was assessed for durum wheat variety targeting in Algeria, where genotype responses to test sites or new sites were predicted as a function of long-

term values in a GIS of winter mean temperature and rainfall over the season, which were selected as covariates for both factorial regression and in a multiple regression for predicting the site PC 1 score (Annicchiarico, Bellah and Chiari, 2006). Data for test sites in a validation data set revealed that GIS-based recommendations implied just a slight yield decrease relative to those based on conventional modelling (Table 20.1), while greatly enlarging the scope for site-specific targeting.

20.2.7 Taking account of yield stability

Static and dynamic stability concepts were introduced in Section 20.1. A few measures of static stability will be considered herein, reflecting the following advantages of static measures over dynamic ones: (i) somewhat higher repeatability or heritability; (ii) estimation independent from the set of tested genotypes (which allows for broader generalization); (iii) less ambiguous agronomic interpretation; and (iv) relevance for increasing food security or agricultural income (Lin, Binns and Lefkovitch, 1986; Annicchiarico, 2002a: 81). The repeatability across different sets of environments may vary, depending on the crop and the region. It increases with the temporal scale of the assessment, but remains distinctly lower than that of genotype mean yield across environments. The assessment of yield stability requires numerous test environments (eight or more) to be reliable, given its high sampling error (Kang, 1998).

The entry regression as a function of environment or year mean yield (Figure 20.1) may have various drawbacks as a stability measure (poor ability to describe G×E effects; too few years to assess stability over time). Lin and Binns' (1988) measure of average within-site temporal stability across years (or crop cycles) is the MS for year within location ($M_{y(l)}$) in an ANOVA limited to location–year cell means of the relevant genotype that also includes the location factor. High stability is indicated by low $M_{y(l)}$. However, the estimate provided by $M_{y(l)}$ is inflated by the experimental error variance. An unbiased estimate can be provided by the temporal stability variance $S_{y(l)}^2$ (Annicchiarico, 2002a: 81):

$$S_{y(l)}^2 = M_{y(l)} - (M_e / r)$$

where M_e = pooled error in the general ANOVA (performed earlier according to Figure 20.2), and r = number of experiment replications. $S_{y(l)}^2$ and $M_{y(l)}$ are equivalent for ranking genotypes, but the former is recommended for testing genotype differences and for adoption in yield reliability indexes. Well-performing genotypes can be compared for $S_{y(l)}^2$ value by ordinary tests for variance comparison such as Fisher's bilateral test (for two entries) or Hartley's test (for more entries), preferably using less critical Type 1 error rates (e.g. $P < 0.05$ or $P < 0.10$). For l test locations and y test years, DF = $l\,(y - 1)$ for each estimated $S_{y(l)}^2$ parameter.

The environmental variance (S^2) measures the static stability across environments. With reference to notations in Section 20.2.2 and for e = number of environments, its value for the genotype i is: $S_i^2 = \sum (R_{ij} - m_i)^2 / (e - 1)$. Greatest stability is $S^2 = 0$. This measure is simpler to compute than $S_{y(l)}^2$ but requires a more complex procedure for genotype comparison based on Ekbohm's test (described in Annicchiarico, 2002a: 82) or other tests.

Temporal stability, which meets farmer's perception of stability, is relevant for site-specific targeting of cultivars (path 4 in Figure 20.2) and can be used also for wide targeting (path 2 in Figure 20.2). The

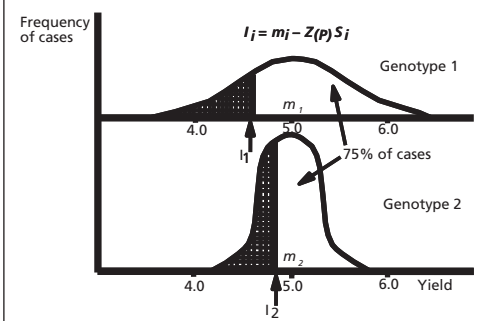
environmental variance is preferable for wide targeting when considering that G×L effects, although not directly relevant to farmers, do influence the consistency of response and the value for farmers of the cultivars across the region. If high-yielding genotypes differ for the relevant stability measure, yield stability can be accounted for by targeting genotypes according to their yield reliability instead of their yield response (Figure 20.2).

Kataoka (1963) proposed a simple index of reliability of yield (or other economic variable) that accounts for the environmental variance. This index estimates the lowest genotype yield that is expected for a probability P fixed according to the level of farmers' risk aversion. For example, P = 0.95 (lowest yield expected in 95 percent of cases) indicates high concern for disastrous events, i.e. marked risk aversion, with little consideration for mean yield response. P may vary from 0.95 (for subsistence agriculture in unfavourable regions) to 0.70 (for modern agriculture in very favourable regions) (Eskridge, 1990). For the genotype i, the index is:

$$I_i = m_i - Z_{(P)}\, S_i$$

where m_i = mean yield, and S_i = square root of the environmental variance, are parameters of the distribution of genotype yields as estimated from the sample of entry yield values; and $Z_{(P)}$ = percentile from the standard normal distribution for which the cumulative distribution function reaches the value P ($Z_{(P)}$ is 0.675 for P = 0.75, 0.840 for P = 0.80, 1.040 for P = 0.85, 1.280 for P = 0.90, and 1.645 for P = 0.95). For the two hypothetical cultivars with same mean yield and different stability reported in Figure 20.7, the advantage in yield reliability of the more stable-yielding cultivar 2 over cultivar 1 is nil when ignoring the stability characteristics ($Z_{(P)}$ = 0), sizeable for the considered level of risk aversion ($Z_{(P)}$ = 0.675), and widening for increasing levels of risk aversion. The same approach may be used for taking account of temporal stability only in the wide targeting of cultivars over the region, substituting the square root of the temporal stability variance ($S_{y(l)i}$) in place of S_i in the index formula for genotype i. Kataoka's approach has been extended to derive indexes also for measures of dynamic stability (Eskridge, 1990). For only two compared genotypes, an alternative measure of reliability of one cultivar is its estimated probability to outperform the other entry (Piepho, 1998).

FIGURE 20.7
Frequency distribution of yield values across environments of two genotypes having same mean yield (m_i) and contrasting yield stability as measured by the square root of the environmental variance (S_i), and genotype yield reliability (I_i) as lowest yield that is expected in 75 percent of cases



The site-specific yield responses of genotypes as modelled by analysis of adaptation can take account of the temporal stability of genotypes (Annicchiarico, 2002a: 85). The adaptive responses are estimates of the mean value of yield or nominal yield that is expected for each genotype on each site. This value is affected by year-to-year varia-

tion in proportion to the level of instability over time of the entry. Imposing a yield reliability assessment basically requires the estimation of a lower confidence bound for each response that depends on its variation in time and the specified level of risk aversion. The following procedure provides, for a modest level of calculation, an approximate solution that may apply to any previous formula for calculating yields or nominal yields from estimates of genotype mean yield (m_i) and interaction parameters (Sections 20.2.3 through 20.2.5). For the genotype i, it suffices to substitute ($m_i - Z_{(P)}$ $S_{y(l)i}$), where $S_{y(l)i}$ and $Z_{(P)}$ corresponds to previous notations, for m_i. For example, for the AMMI-1 model:

$$N_{ij}' = m_i - (1.28\ S_{y(l)i}) + (u_{i1}'\ v_{j1}')$$

where N_{ij}' = nominal yield reliability of the genotype i at the site j, when estimating the lowest response expected in 90 percent of cases ($Z_{(P)} = 1.28$). Compared with nominal yields (relative to mean responses, i.e. $Z_{(P)} = 0$), the AMMI-1 responses for nominal yield reliability are parallel but lowered to an extent that is modest for stable material and severe for unstable one (possibly modifying the crossover points between top-ranking entries that determine the limits of subregions). This simple approach for modelling yield reliabilities assumes that the year-to-year variation for entry yield on each site is substantially constant across the range of site mean yields (joint regression), PC scores (AMMI) or covariate values (factorial regression). This assumption may hold even when site mean yields vary widely (Annicchiarico, 2002a: 86), because unfavourable sites frequently display large temporal variation of genotype yields due to wide year-to-year extent of climatic stresses. Otherwise, a data transformation could be envisaged (Annicchiarico, 2002a p. 86).

Information on the most reliable genotypes on each site may be extended to new sites nearly as described in Section 20.2.6, the only difference concerning the introduction of the term ($- Z_{(P)}\ S_{y(l)i}$) in formulae for estimating the entry yields or nominal yields on new sites.

20.3 DEFINING A BREEDING STRATEGY

20.3.1 Overview

Global-oriented or large international breeding programmes are forced to identify some transcontinental or transnational subregions (within which to breed for wide adaptation). For breeding programmes targeting a medium-sized region, a specific-adaptation strategy may (e.g. Ceccarelli, Grando and Impigli, 1998; Annicchiarico, Bellah and Chiari, 2005; Annicchiarico, 2007) or may not (e.g. Singh *et al.*, 1992) increase the overall selection gains. When convenient, this strategy helps national breeding programmes withstand the increasing competition exerted in national markets by international seed companies (by exploiting G×L effects at a scale inaccessible for these companies). In addition, it enhances the security of food production or agricultural income in a sustainable manner, by: (i) fitting cultivars to less favoured environments, instead of altering these environments (possibly with costly or environment-unfriendly inputs) to fit widely-adapted cultivars; and (ii) safeguarding the diversity of cultivated material. Finally, it may facilitate the technological adaptation of varieties, by fixing characteristics of specific interest to subregions.

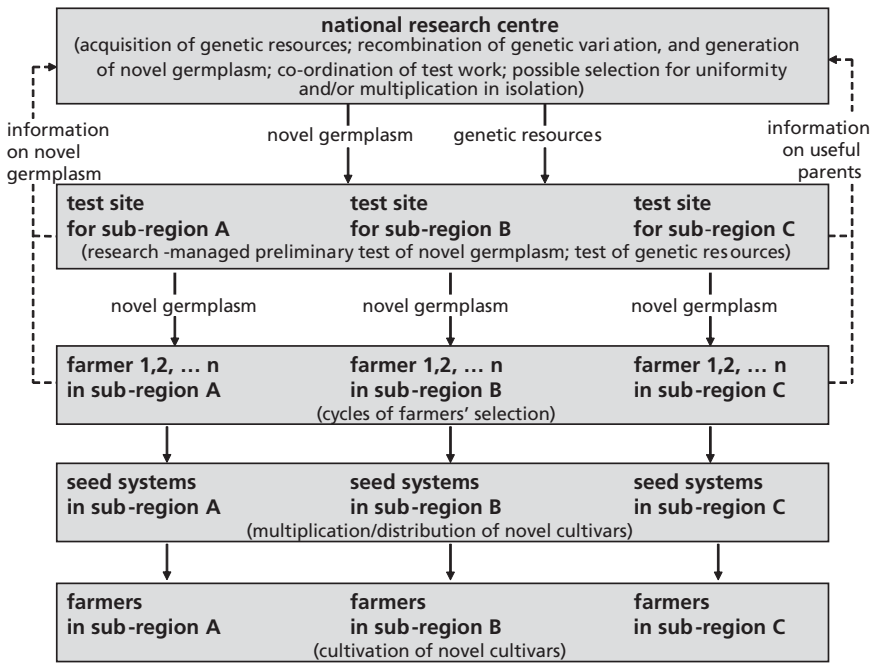
Breeding programmes of neighbouring countries that share a similar diversity of target environments could cooperate to share resources and become, on the whole,

more cost-efficient. Their cooperation might widen the scope for a specific-adaptation strategy, by enlarging the size of possible subregions (e.g. a transnational drought-prone area). International research centres could contribute to this strategy by selecting segregating material specifically adapted to distinct transnational agro-ecological zones (Ceccarelli, 1996). The size of non-repeatable G×L effects and practical considerations usually limit to two or three the number of subregions that a national programme or a few cooperating ones might possibly target.

Despite its potential interest, increased yield stability has usually been a minor breeding target. The adoption of variety types with high levels of heterozygosity and/or heterogeneity has been limited by the fewer opportunities for maximizing the yield potential that such types may offer and, sometimes, by the difficulty of meeting the uniformity level set for variety registration by many national legislations. Within a given variety type, the selection is hindered by the modest heritability and repeatability of the stability trait and the fairly high number of environments required for a reliable estimation.

When the target region is subject to large G×E interactions, effective breeding requires MET. Breeding for specific adaptation and yield stability provides an ideal framework for linking formal breeding with participatory plant breeding and overcoming the limits of each individual approach. In particular, the availability of farmers' selection environments can overcome the difficulty of formal selection programmes to adequately sample the different target environments and variety users. A national (or regional) research centre that serves the different subregions (Figure 20.8) could centralize the following tasks that participatory plant breeding could hardly assume: (i) performing sufficient crossing and hybridization work to produce largely diversified, possibly subregion-specific, novel germplasm; (ii) producing in isolation large and broadly-based composites (which is very important for outbred crops; Witcombe, 2001); and (iii) coordinating the testing of novel germplasm and large collections of genetic resources through advanced procedures that minimize the micro-environmental variation and, for novel germplasm, allow for the combined analysis of entry yields across unreplicated and possibly largely unbalanced farmers' trials (which is particularly important for inbred crops; Atlin, Cooper and Bjørnstad, 2001). This centre may also perform a preliminary screening of novel germplasm and genetic resources, while leaving the main early selection stage(s) and the main screening of genetic resources as a researcher-managed activity performed in one representative site for each subregion (the national centre might, or might not, also act as one such site). The overall selection scheme varies depending on the crop breeding system, e.g. including the farmer-managed selection among advanced lines produced by single-seed descent or among and within bulks (derived from different crosses or F_2 plants) for inbreds, and the phenotypic selection between and within composite populations for outbreds. The genotypic selection of outbreds for each subregion (e.g. based on half-sib progenies) could mostly be only research-managed. The selected varieties might enter the local seed systems (e.g. multiplied and traded by farmers-entrepreneurs), the formal seed systems, or both (e.g. after selection for uniformity prior to official registration) (Figure 20.8). In the absence of participatory breeding, specific selection is mainly performed at the site representative of a given subregion, but might be complemented

FIGURE 20.8
A specific-adaptation strategy implying the generation of novel germplasm in a national (or regional) research centre, the research-managed early selection of novel germplasm and evaluation of genetic resources in distinct subregions, and subsequent farmers' selection stages in each subregion



by within-subregion on-farm evaluation of candidate varieties.

Identifying optimal selection environments is a basic element of the breeding strategy. It is of paramount importance when selecting for wide adaptation in the presence of large G×E effects, where selection should be devised across environments that contrast for these effects and are jointly able to reproduce the genotype mean responses over the region. Optimal selection environments are of crucial importance in all cases for recurrent selection or genotypic selection of outbreds, which are severely constrained by the small number of possibly usable environments.

Sometimes, selection may partly be performed in managed or artificial environments instead of agricultural sites. These environments differ for one or more environmental factors strictly related to G×L (or G×E) interaction occurrence, and reproduce the factor levels that feature different subregions for specific adaptation or contrasting sites (or environments) for wide adaptation.

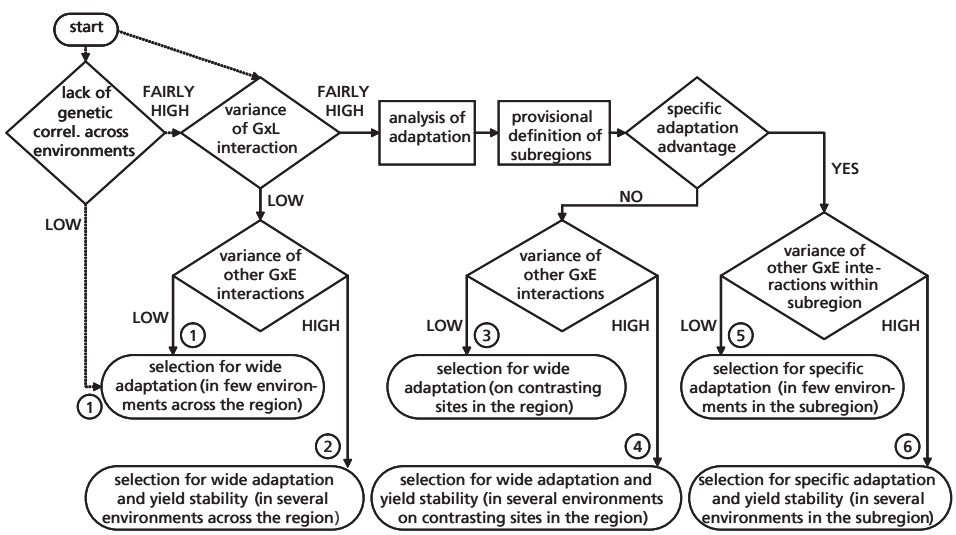
MET data that sample adequately the possible genetic base and the target environments may help breeding programmes to define a strategy in relation to G×E interactions. Its elements include the adaptation strategy, the yield stability targets, the selection environments and, possibly, the

parent material, the genetic structure, the ideotype and the single adaptive traits, and the role of participatory plant breeding. Decisions on most of these elements may conveniently be verified by further experimental work. In particular, wide- vs. specific-adaptation strategies may be compared on the basis of actual yield gains. A fair comparison of adaptation strategies implies similar costs by assuming the same total number of selection environments. Specific adaptation may then allow for higher or lower yield gains than wide adaptation, because the advantage of exploiting the portion of G×L effects that relates to subdivision of the target region (i.e. the genotype × subregion interaction) may be offset by the greater error of the estimated entry means that arises from the lower number of selection environments within each subregion (the error depends mainly on the size of G×E interaction over the region for wide adaptation and within each subregion for specific adaptation: see Section 20.3.4).

For targeting genotypes, only the G×L effects that imply a change of top-ranking genotype(s) across locations are relevant (as these effects define the subregions). For defining the adaptation strategy, all G×L effects that arise from lack of genetic correlation among sites for entry response are relevant, because the results for a given data set are extrapolated to produce information on the G×L effects that are likely to be met in future breeding for the region. A candidate subregion includes the locations that are similar in terms of genetic correlation.

The main analytical steps to provisionally define the adaptation strategy and yield stability targets from multi-location yield trials repeated over time are summarized in Figure 20.9. G×L or G×E effects that arise from heterogeneity of genotypic variance

FIGURE 20.9
Flow chart of steps for defining the adaptation strategy and yield stability targets from analysis of multi-location yield trials repeated over time



NOTES: G×L = genotype × location; G×E = genotype × environment (environment as location–year or location–crop cycle combination).

rather than lack of genetic correlation among environments are irrelevant for breeding (as they merely modify the size of the entry differences) and should be removed if they are too large. The relative size of the lack of genetic correlation and the heterogeneity of genotypic variance among environments may be estimated or, more simply, the need for transforming data could be verified (see Section 20.3.2), before estimating the variance components relative to spatial and temporal G×E interaction. An analysis of adaptation aimed to define candidate subregions (by classifying test sites on the basis of their similarity for G×L effects) may be justified if the G×L interaction variance is significant and moderately large relative to the genotypic variance, e.g. ≥30–35 percent (Atlin *et al*., 2000). Two (or possibly three) candidate subregions may be identified that are large enough to be of practical interest and lend themselves to a definition based on geography, environmental factors or farming practices. Wide- and specific-adaptation scenarios can be compared in terms of yield gains predicted from original yield data of the same data set. Wide adaptation may be preferred owing to low G×L interaction variance or to high G×L interaction variance with no clear advantage of specific breeding, with different implications for the choice of selection environments (the analysis can also help locate these environments). Adaptation strategies may also be compared according to predicted gains in other data sets (e.g. including a few sites representative of the candidate subregions), or actual gains from following selection work.

Yield stability may be justified as a target when the overall variance accounted for by the relevant G×E interaction components (either the G×Y plus G×L×Y interaction or the within-site G×Y interaction, depending on the ANOVA model) is large relative to the genotypic variance component (e.g. ≥200 percent). Decisions on the stability target are subregion-specific (depending on results for the relevant subset of sites) and can be affected by other elements (e.g. costs of additional selection environments; emphasis on food security policies).

20.3.2 Types of data and estimation of variance components

The current requirements for use of data sets are more stringent than those for targeting genotypes, given the larger inference space of the analysis. Ideally, the sample of sites should represent the different areas and cropping systems in proportion to their importance, and the germplasm sample should represent the genetic base of local interest (by including the main cultivar types and origins, or breeding lines derived from recombination from the major germplasm groups). The lack of random sampling of entries is not a limitation, because a set of elite varieties or breeding lines may represent the genetic base better than a random sample.

The G×E interaction variance components relative to the lack of genetic correlation and the heterogeneity of genotypic variance among environments can be estimated as described by Cooper, DeLacy and Basford (1996). If the latter term has larger variance than the former, it should be reduced by a suitable data transformation. This may occur when the environment mean yields vary widely (Annicchiarico, 2002a: 51). In this situation, the regression of the within-site phenotypic variance of genotype yields (averaged over test years and replications) as a function of site mean yield, with both terms expressed on a logarithmic scale, provides a quick option for verifying the need for transforming data and indicates the proper transformation (Annicchiarico,

2002a: 53). The regression slope $b \approx 2$ suggests a logarithmic transformation; $b \approx 1$ suggests a square root transformation; b not statistically significant from 0 discourages any transformation.

The reference ANOVA models are those reported in Section 20.2.2, holding genotype, location and year as random factors. The model without year factor is relevant also for estimating the variance components for genotype and G×E interaction (as required in Section 20.3.4), upon substitution of environment for the location factor. Adopting a Restricted Maximum Likelihood (REML) method allows to estimate variance components also for unbalanced data sets (Patterson, 1997).

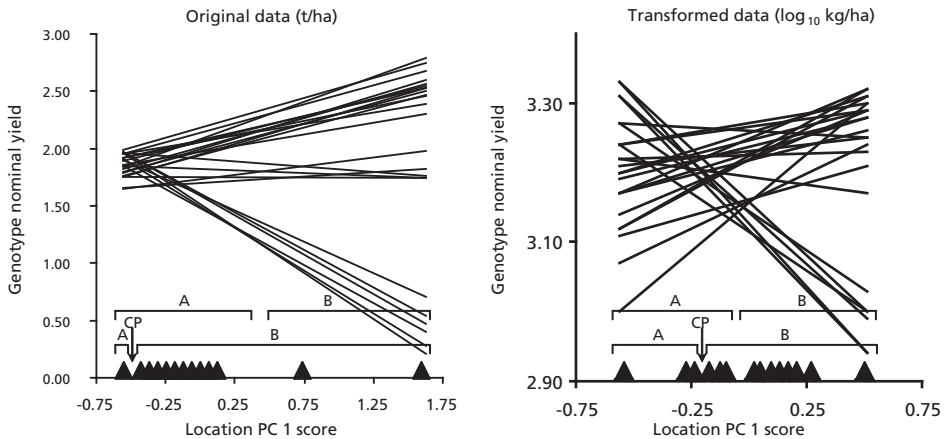
20.3.3 Classifying test sites and defining candidate subregions

Classifying test locations according to their similarity for G×L effects is needed to empirically identify candidate subregions for specific breeding, and is useful, especially in a wide-adaptation prospect, to locate selection environments. There are many possible classification methods (DeLacy *et al.*, 1996a; Annicchiarico, 2002a). Pattern analysis in its classification mode implies the hierarchical cluster analysis of sites performed on the matrix of genotype by location original yields (averaged across test years and replications) standardized within location to zero mean and unit standard deviation (thereby eliminating also the possible effect of heterogeneity of genotypic variance among sites), using a squared Euclidean distance as the dissimilarity measure and Ward's clustering method (DeLacy *et al.*, 1996a). This analysis is rapid (through freely-available software) and has the theoretical advantage of providing a grouping of sites that reflects the opportunities for exploiting indirect selection responses to locations (as these responses are maximized within potential subregions and minimized across subregions; Cooper *et al.*, 1996). It also allows for jointly analysing different data sets (e.g. relative to different test years) that have several common sites but few or no common entries, using a procedure proposed by DeLacy *et al.* (1996b).

Site classification may also follow the site ordination by modelling techniques (performed on transformed data, when appropriate). The cluster analysis of locations—using Ward's method—may be carried out on the site characteristics affecting the modelled adaptive responses, namely, the mean yield for joint regression, the significant PC scores for AMMI, and the significant covariates for factorial regression. No standardization of PC score variables is required, because the variation in site score on each PC axis is proportional to the importance of each variable. This approach has the theoretical advantage of assessing the site similarity after reducing the noise portion in the G×L interaction matrix [with the same aim, Crossa *et al.* (1991) suggested to submit the G×L matrix of AMMI-modelled yields to pattern analysis]. AMMI + cluster analysis also offers the opportunity to quickly pool the results of two or more data sets that have same test locations and different genotypes, by performing the cluster analysis on site scores of all significant PC axis issued by AMMI analyses of the different data sets (Annicchiarico *et al.*, 2006).

A truncation criterion for cluster analysis by any of the above techniques may be the lack of significant G×L interaction (at a given P level) within a group of sites (going backward from the last fusion stage to the formation of groups, and performing an ANOVA for each newly-formed group on

FIGURE 20.10
Nominal yields of durum wheat genotypes as a function of the scaled G×L interaction PC 1 score of 14 Algerian locations (black triangles), and definition of two subregions (A and B) based on cluster analysis of site PC 1 scores (above) and the estimation of the main crossover point *CP* (below), for original and log-transformed data



NOTE: Based on data by Annicchiarico, Bellah and Chiari, 2005.

data from the member sites). Other criteria are also available (DeLacy *et al.*, 1996a). In most cases, the maximum number of subregions (two or three) is fixed *a priori*.

Singh, Ceccarelli and Grando, (1999) proposed the estimation of the main crossover point in the joint regression model, i.e. the site mean yield where the interaction of crossover type between genotypes reaches the highest frequency, as a cut-off for dividing the test sites into two subregions (one high- and one low-yielding). This approach was extended to AMMI-1 and to one-covariate factorial regression models by Annicchiarico (2002a).

The cited techniques often provide different classification results for the same data. Their empirical comparison based on the assumption that a useful technique tends to maximize the selection gains for a specific-adaptation strategy suggested the superiority of pattern analysis and AMMI + cluster analysis over the other methods (based on cluster analysis or the main crossover point), while confirming the need for modelling transformed data when most G×E effects are due to heterogeneity of genotypic variance among sites (Annicchiarico, 2002b). Figure 20.10 provides some insight into reasons for these results. Responses for original yields implied larger G×L effects for higher-yielding sites (here tending to higher PC 1 score), owing to their higher genotypic variance. As a consequence, many low-yielding sites were grouped together by cluster analysis, because they tended to appear less distinct than they really were. The data transformation (selected as outlined in Section 20.3.2) removed the heterogeneity of genotypic variance between low- and high-yielding sites (while maintaining the lower genotype variance for sites with intermediate PC 1 score that is intrinsic to the adaptive responses). Cluster

analysis, unlike the main crossover criterion, subdivided the sites on the basis of their discontinuities for G×L effects as expressed by the relevant characteristic, i.e. the site PC 1 score, thereby justifying its better classification ability.

A two-subregion scenario is the first to compare with wide adaptation, as its lower interest relative to wide adaptation rules out more complex specific-adaptation scenarios. A small subregion that hardly justifies any specific breeding may be merged with the most similar large subregion (unless farmers have very specific and different preferences). An indication of the approximate proportion of the target region occupied by each subregion is useful for comparing the adaptation strategies and possibly for other reasons (e.g. estimation of seed markets). A very rough indication is provided by the proportion of test sites assigned to each subregion in the analysis of adaptation. The scaling-up of subregion definition over the region may be somewhat arbitrary, by assigning an area represented by a given test site to the subregion in which the test site was classified, or characterizing the subregions according to their mean values for environmental variables correlated with G×L interaction PC scores of sites (e.g. Annicchiarico, 1992). These variables may also be exploited for a discriminant analysis of subregions which may provide a thorough up-scaling procedure and, for the frequent case of two subregions, can easily be performed by multiple regression (Annicchiarico, 2002a: 59). The possible interfacing of this analysis with GIS allows for a detailed geographical definition of subregions (Annicchiarico, Bellah and Chiari, 2005). As an alternative, a few well-performing reference genotypes characterized by contrasting adaptation and a specific ranking order in each subregion could be used to indirectly characterize new sites on the basis of their response in small trials (Fox and Rosielle, 1982). For example, the cultivars 'La Rocca', 'Prosementi' and 'Orchesienne' in Figure 20.4(A) could be used for assigning new sites to one of three subregions identified by AMMI + cluster analysis. Additional information may allow estimating the relative size of subregions in terms of crop growing area or, especially for private companies, seed market importance.

The assessment of site similarity for G×L effects is recommended to explore the opportunities for specific breeding. It revealed, for example, that winter cold stress is more important than drought stress in determining subregions for winter cereals in Italy (Annicchiarico, 1997) and Algeria (Annicchiarico, Bellah and Chiari, 2005). Sometimes, however, candidate subregions for comparing adaptation strategies are defined *a priori* on a geographical, climatic or crop management basis (e.g. Atlin *et al*., 2000). The investigation of environment similarity for G×E effects in fairly small data sets including a few test sites representative of distinct geoclimatic areas and several environments per site (issued by different test years and, possibly, different crop managements per year) may provide useful indications for the adaptation strategy (e.g. Annicchiarico and Iannucci, 2008), as specific breeding requires that location is the main determinant of environment classification and crossover G×E interactions.

20.3.4 Comparison of wide- vs. specific-adaptation strategies

The comparison may concern predicted or actual yield gains, and vary depending on the crop breeding system and the selection procedures. It hypothesizes one selection cycle (over a definite time span) on research-managed selection sites or, possibly, managed environments, but more

selection cycles may be envisaged, especially for actual yield gains. The adaptation strategies imply similar costs by assuming the same total number of selection environments (as number of sites × number of years), assigning sites to subregions roughly in proportion to their relative size. Specific selection is based on entry mean yields over selection environments of the target subregion. Selection for wide adaptation is based on entry mean yields over all test environments hypothesized for specific breeding, in agreement with Lin and Butler's (1988) suggestion to choose selection sites across the region in a stratified manner and in proportion to the relative size of site groups.

The following method (Annicchiarico, 2002b) aims to compare adaptation strategies for inbred lines or clones in terms of predicted yield gains over the target region from undefined selection sites, using original yields of a data set possibly used also for defining candidate subregions. In general, the average predicted yield gain over E environments can be estimated as (DeLacy *et al.*, 1996a): $\Delta G = i\, h^2\, s_p$ (where i = standardized selection differential, h^2 = estimated broad sense heritability on a genotype mean basis, and s_p = square root of the estimated phenotypic variance across environments). In particular:

$$h^2 = s_g^2 / [s_g^2 + (s_{ge}^2 / E) + (s_e^2 / E\, R)] \quad [20.1]$$

where s_g^2, s_{ge}^2 and s_e^2 are estimates of the variance components for genotype, G×E interaction and pooled experimental error, respectively, and E and R = numbers of selection environments and experiment replications. The s_p term is equal to the square root of the denominator in equation [20.1]. In the formulae for prediction of yield gains, h^2 values are calculated from variance components estimated by a REML method for genotype and environment as random factors using all test environments (for wide adaptation) or their subsets (for specific adaptation), fixing E and R as hypothesized for selection. R and i are set to constant values in the assessment. Hereafter, E_A and E_B represent the number of selection environments for two subregions (A and B, respectively) in a specific-adaptation scenario, whereas $E_{AB} = E_A + E_B$ is the number of selection environments that are used, in a wide-adaptation scenario, for parallel selection across the subregions. P_A and P_B represent the proportion of the target region occupied by subregions A and B ($P_A + P_B = 1$), as estimated by the proportion of test sites classified in each subregion or, more precisely, after scaling up the subregion definition (for private companies, they might rather estimate the relative commercial importance of the subregions). E_A and E_B should be roughly proportional to P_A and P_B, respectively (e.g. for $E_{AB} = 6 = 3$ sites by 2 years, and $P_A = 0.64$: $E_A = 4 = 2$ sites by 2 years, and $E_B = 2 = 1$ site by 2 years). The average predicted gain (per unit area) by a wide-adaptation strategy is:

$$\Delta G_W = i\, h_{AB}^2\, s_{p(AB)} \quad [20.2]$$

where h_{AB}^2 and $s_{p(AB)}$ are obtained from equation [20.1] after estimating the components of variance for the whole set of environments in the data set, and inserting E_{AB} and R values as appropriate. The average predicted yield gain over the region provided by breeding for specific adaptation (ΔG_S) arises from a weighted mean of the gains ΔG_A and ΔG_B predicted for the subregions A and B, respectively:

$$\Delta G_A = i\, h_A^2\, s_{p(A)}\ ;\ \Delta G_B = i\, h_B^2\, s_{p(B)}\ ;$$
$$\Delta G_S = [(\Delta G_A\, P_A) + (\Delta G_B\, P_B)] / (P_A + P_B) =$$
$$(\Delta G_A\, P_A) + (\Delta G_B\, P_B) \quad [20.3]$$

where heritability and phenotypic variance values are obtained from equation [20.1] after estimating the components of variance for the subset of test environments belonging to subregion A (values h_A^2 and $s_{p(A)}$) or B (h_B^2 and $s_{p(B)}$), and inserting E_A or E_B and R in the equations as appropriate. This procedure can easily be extended to three (or more) subregions, computing the specific-adaptation gains across more than two subregions. Another procedure for the same context (i.e. predicted yield gains for inbred lines or clones from undefined selection sites) was proposed by Atlin *et al*. (2000). Piepho and Möhring (2005) expanded this approach by considering a more complex scenario that maximizes the selection gains by using for specific selection also the data from other subregions. These data are given a weight proportional to their relevance for the target subregion.

Another adaptation strategy may contemplate the selection in one subregion also for another subregion, so that the yield gains in the latter subregion derive from correlated responses in an indirect selection context. Formulae for comparing this strategy against the previous ones in terms of predicted gains (taking conveniently into account the effect of G×E interactions within subregions, unlike more simplified approaches) are reported elsewhere (Annicchiarico, 2002a: 68; 2002b).

For open-pollinated species, the comparison of strategies based on predicted gains from selection of populations (as represented by cultivars in a MET data set) is frequently out of context, as selection mainly concerns individual plants. Preliminary indications may be obtained by comparing the top-ranking cultivars according to each adaptation strategy, as if each cultivar was used as the unique genetic base and could provide, when chosen, a constant gain from intra-population selection (e.g. Annicchiarico and Piano, 2005). Predicted gains could be computed as the difference between the top-ranking cultivar and the mean of all tested entries.

Reliable predicted gains for comparing adaptation strategies in open-pollinated species may be obtained from multi-environment progeny testing of individuals as half-sib or full-sib families or as selfed progenies. It suffices to substitute the appropriate narrow sense heritability term (Wricke and Weber, 1986) for the broad sense heritability in formula [20.1] when using this equation for estimating ΔG_W and ΔG_S according to formulae [20.2] and [20.3]. For example, the following equation and the square root of its denominator allow for estimating h^2 and s_p, respectively, for half-sib progeny testing targeted to selection of parent material as clone or selfed progeny:

$$h^2 = 0.5\, s_a^2 / [0.25\, s_a^2 + (0.25\, s_{ae}^2 / E) + (s_e^2 / E\, R)] \qquad [20.4]$$

where s_a^2, s_{ae}^2 and s_e^2 are estimated variance components relative to the additive genetic variance, the interaction of additive genetic effects with environment and the pooled error, respectively, and E and R = numbers of hypothesized selection environments and experiment replications. The REML analysis performed on family plot values for the relevant sets of test environments provides the estimate of s_e^2, and allow estimation of the other variance components from the variance among families (s_g^2) and the family × environment interaction variance (s_{ge}^2) (assuming no inbreeding in the tested material) as:

$$s_a^2 = 4\, s_g^2;\ s_{ae}^2 = 4\, s_{ge}^2.$$

The previous procedures assume undefined selection sites. Thus, they relate to the average screening ability of sites within each subregion (as estimated from the sample of test sites). It is also possible to compare the adaptation strategies for predicted yield gains from selection in managed environments or in previously-defined, nearly-optimal selection sites (Annicchiarico, Bellah and Chiari, 2005). Predicted gains are correlated gains from the defined selection environments to the target environments. For inbred lines or clones, they are (DeLacy *et al*., 1996a):

$$\Delta G_{T/S} = i\, r_{(S,T)}\, s_{p(T)} \qquad [20.5]$$

where $r_{(S,T)}$ = phenotypic correlation for entry mean yield between selection and target environments, and $s_{p(T)}$ = phenotypic standard deviation in the target environments. The basic difference with previous formulae for predicted gains of inbreds or clones is the substitution of the appropriate $r_{(S,T)}$ term in place of h^2. For agricultural sites, however, the gains relate to estimates of site screening ability that may largely be affected by specific conditions during the test years.

A promising specific-adaptation strategy may be compared with wide adaptation in terms of actual yield gains. Selection is performed both independently within subregion (specific adaptation) and jointly across subregions (wide adaptation) at previously-defined selection sites (or managed environments), contemplating the same total number of selection environments (roughly assigned in proportion to the relative size of subregions). The selected groups are compared across a sample of environments, assessing the gains provided by each strategy in each subregion (e.g. in terms of difference between the group mean and the mean of a set of high-yielding cultivars) and weighting them on the relative importance of the subregions. For example, Ceccarelli, Grando and Impiglia (1998) selected barley genotypes within a large germplasm pool for wide and for specific adaptation to an unfavourable (A) and a favourable (B) subregion, reporting the mean values of the selected groups tested in the two subregions. For one set of material (Cohort 89), the yield gains estimated with respect to a set of high-yielding control cultivars were: ΔG_A = 0.03 t/ha, and ΔG_B = 0.08 t/ha, for specific adaptation; ΔG_A = –0.03 t/ha, and ΔG_B = 0.08 t/ha, for wide adaptation. Based on these values, the advantage of the former strategy is manifest. If the subregions were of equal size ($P_A = P_B$ = 0.50), the gain over the region from specific (ΔG_S) and from wide (ΔG_W) breeding could be estimated as:

$\Delta G_S = (\Delta G_A\, P_A) + (\Delta G_B\, P_B) = (0.03 \times 0.50) + (0.08 \times 0.50) = 0.055$ t/ha per cycle
$\Delta G_W = (\Delta G_A\, P_A) + (\Delta G_B\, P_B) = (-0.03 \times 0.50) + (0.08 \times 0.50) = 0.025$ t/ha per cycle

implying a greater efficiency of specific breeding equal to $\Delta G_S/\Delta G_W = 0.055/0.025 = 220$ percent.

Large data sets for inbred lines or clones that include many entries and several test years may also be used for assessing actual yield gains of adaptation strategies, after defining candidate subregions and selection sites. For example, Annicchiarico, Bellah and Chiari (2005) used two years and a total of three sites of a three-year durum wheat data set for wide or subregion-specific entry selection, and the remaining environments to assess actual gains in each subregion (estimated as yield difference between the mean of selected entries and the mean of all entries), averaging results across the three possible pairs of selection years (Table 20.3).

TABLE 20.3
Mean yield of selected durum wheat entries, and average observed and predicted yield gains per selection cycle in two subregions and over the Algerian durum wheat cropping region, for specific- and wide-adaptation strategies

	Specific adaptation	Wide adaptation	Specific/wide ratio (%)
Mean yield (t/ha) [a b]			
Subregion A	1.899	1.833	103.6
Subregion B	3.031	3.031	100.0
Observed gain (t/ha) [a b c]			
Subregion A	0.233	0.167	139.5
Subregion B	0.372	0.372	100.0
Region	0.327	0.305	107.1
Predicted gain (t/ha) [a]			
Subregion A [d]	0.199	0.181	109.9
Subregion B [d]	0.509	0.505	100.8
Region [d]	0.409	0.400	102.2
Region [e]	0.316	0.304	104.1

[a] Selected fraction: 3 entries out of 24. Total selection environments: 6 (3 sites by 2 years), of which 2 assigned to Subregion A (proportion of the region = 0.322) and 4 to Subregion B (proportion of the region = 0.678).
[b] Values averaged across three pairs of test years.
[c] Gain computed as the difference between the mean of selected entries and the mean of all entries.
[d] For defined selection locations (using equation [20.5]). Values averaged across three pairs of test years.
[e] For undefined selection locations (using equation [20.2] for wide adaptation, and equation [20.3] for specific adaptation; in the latter, ΔGA = 0.161 t/ha and ΔGB = 0.304 t/ha).
Source: Annicchiarico, Bellah and Chiari, 2005.

Gains over the region for each strategy were weighted means of those in each subregion (as shown for the barley data) and indicated the greater efficiency of specific over wide breeding ($\Delta G_S/\Delta G_W$ = 107.1 percent) as a consequence of greater gain (+39.5 percent) in the stressful subregion A. This procedure is less reliable than comparisons for actual gains on a larger and independent genotype sample. However, its results agreed closely with those of predicted gains for undefined selection sites ($\Delta G_S/\Delta G_W$ = 104.1 percent), while being less consistent with those of predicted gains for the same set of selection sites ($\Delta G_S/\Delta G_W$ = 102.2 percent) (Table 20.3).

Especially for cross-pollinated crops, the lack of sufficiently large data sets may limit the comparisons of adaptation strategies based on predicted gains, giving impulse to those based on actual gains. One example was given by Annicchiarico (2007) for phenotypic selection of alfalfa in artificial environments capable of reproducing the adaptive responses occurring across the three subregions shown in Figure 20.4(A). Direct selection for specific adaptation targeted each of the contrasting subregions A and C, exploiting correlated selection gains for the intermediate subregion B. To reduce the evaluation costs, the selection environments also acted as test environments for the selections (possibly introducing some bias relative to agricultural sites).

All cited procedures hypothesize growing the novel germplasm in all selection environments. Their indication of some advantage for specific breeding probably implies larger gains after optimizing other elements of the breeding strategy by considering, at least to some extent: (i) the allocation of novel germplasm to only one

subregion on the basis of crucial adaptive traits (or molecular markers) assessed preliminarily at the main research centre; or (ii) the use of a distinct genetic base for each subregion. Indications for these elements may be provided by the MET data set, with or without further research. Anyway, the comparison of adaptation strategies could not take account of some positive effects of breeding for specific adaptation (Section 20.3.1) that are difficult to quantify.

20.3.5 Definition and use of selection environments

In the presence of sizable G×L interaction, the main research centre may host a preliminary selection stage if its screening ability for the target region is high. According to formula [20.5] in Section 20.3.4, the screening ability of a site (or a managed environment) is proportional to the phenotypic correlation between entry yields on the site and entry mean yields over the target environments. The phenotypic correlation takes account of the genetic correlation between selection and target environments and the broad sense heritability on the site (Cooper, DeLacy and Basford, 1996). When breeding for specific adaptation, this preliminary selection stage may also allow for the allocation of material to a specific subregion on the basis of adaptive traits.

Multi-environment data can also help locate optimal selection sites for research-managed selection (also usable for detecting parent germplasm of specific interest for subregions; see Figure 20.8). Preliminary indications may be obtained from site ordination in the analysis of adaptation or site classification for G×L effects. The optimal selection site for a given subregion has the highest screening ability for the relevant target environments. When adopting more sites within a subregion, it is the joint screening ability of the sites (as indicated by phenotypic correlations between selection and target environments for entry yields) that should be maximized (e.g. Annicchiarico, Bellah and Chiari, 2005).

Selection for wide adaptation in the presence of sizable G×L interaction should be performed across sites that contrast for G×L effects (as hypothesized for comparing adaptation strategies) and are jointly capable of maximizing the screening ability (as indicated by phenotypic correlations), rather than across sites that maximize individually the screening ability (which are implicitly similar for G×L effects). Contrasting sites offer the opportunity for disclosing and selecting material capable of assembling different adaptive traits of interest for the region (Calhoun *et al*., 1994). Thus, optimal selection sites may be identified for wide or specific adaptation by the same procedures. Phenotypic correlations between selection and target environments also allow the assessing of the lower yield gain expected from adopting suboptimal sites.

Managed or artificial selection environments that reproduce the genotype adaptive responses and do not imply very high implementation costs can partly replace agricultural selection sites to reduce costs (especially when optimal sites belong to remote areas or have little infrastructure) or to increase the selection gains (especially when agricultural sites are subject to wide G×Y interaction due to unpredictable climatic conditions). For example, the artificial environments in Figure 20.4(B) were established on the ground of the positive correlations of soil clay content and drought stress level with PC 1 score of alfalfa test sites in Figure 20.4(A) (Annicchiarico, 1992). They could reproduce the adaptive responses occurring in three subregions (as shown by three reference varieties: Figure 20.4),

and may be used to select for wide or specific adaptation (Annicchiarico, 2007). Selection under the natural conditions of the breeding centre (located in subregion A), implying sandy-loam soil and negligible stress, would produce varieties specifically adapted to these conditions, such as cultivar 'Lodi' in Figure 20.4(B).

Managed environments may also be used to breed for wide adaptation to regions featured by large within-site G×Y interaction and small repeatable G×L effects due to wide year-to-year climatic variation. In such regions, agricultural sites in individual years frequently misrepresent the target environments, leading to low selection gains (Cooper, DeLacy and Basford, 1996). An optimal set of managed environments can be identified by assessing the joint screening ability of these environments (Cooper *et al*., 1995, 1997). Federer and Scully (1993) proposed statistical designs to select material for wide adaptation across a factorial combination of two or three crop management or physical factors that reproduce the variation for environmental variables associated with G×E effects.

Selecting on agricultural sites for wide adaptation to climatically unpredictable regions may increase its efficiency by a procedure proposed by Podlich, Cooper and Basford (1999). A large sample of target environments is classified on the basis of G×E interaction effects, identifying a few major groups whose relative frequency is estimated and that are characterized either by a specific response of some probe genotypes or a definite value of some crucial climatic variable(s). Each new selection environment is classified according to the response of the probe genotypes (grown along with the tested material) or the relevant climatic variable(s), and is given a weight on the future MET-based entry selection that is proportional to the frequency of its group.

The optimal number of selection sites, years and experiment replications for inbred lines of clones in a region or subregion may be investigated after estimating the genotypic and genotype-environmental variance components for a representative sample of elite material and target environments. The aim is maximizing, for about same costs, the yield gain: $\Delta G = i\, h^2\, s_p$, where h^2 and s_p are computed by the following equation and the square root of its denominator, respectively, for L locations, Y years and R replication numbers hypothesized for selection (Cooper *et al*., 1999):

$$h^2 = s_g^2 / (s_g^2 + s_{gl}^2 / L + s_{gy}^2 / Y + s_{gly}^2 / LY + s_e^2 / RLY)$$

Estimates may also relate to more complex scenarios, e.g. two-stage selection (Grüneberg *et al*., 2004) or among-cross (bulk) plus within-cross pure line selection (Cooper *et al*., 1999). Research-managed selection trials would usually include at least two replications, while the number of selection years is kept low (often no more than two) so as not to delay the release of varieties. Thus, decisions mainly regard the number of selection sites. Selecting also for yield stability may lead to increases in this number (Figure 20.9), if socio-economically convenient.

Predicted gains for different scenarios relative to managed environments depend on the h^2 value over selection environments (as affected by hypothesized L, Y and R values) and the genetic correlation between selection and target environments (DeLacy *et al*., 1996a; Qiao *et al*., 2004).

In various contexts, only an unbalanced data set (with possibly many missing genotype-environment cell means) may be

available for entry selection. Best Linear Unbiased Prediction (BLUP) entry means as estimated by a REML method should be used in this case. BLUP entry main effects (P_i) differ from Best Linear Unbiased Estimate (BLUE) entry main effects (G_i, as provided by least squares means) because they are shrunk to a greater extent for entries with less observations, to take account of the greater uncertainty introduced by less MET. In particular, P_i effects are shrunk in proportion to the difference to unity of the broad sense heritability on an entry mean basis (DeLacy *et al.*, 1996a), i.e. $P_i = (h^2\ G_i)$, where $h^2 = s_g^2 / [s_g^2 + (s_{ge}^2 / e_i) + (s_e^2 / e_i\ r_i)]$, and e_i and r_i are numbers of environments and experiment replications for the entry i (the latter as harmonic mean). The variance components are constant values estimated by a REML method for the entire data set. This procedure is simpler and more reliable than other methods that also consider the broad sense heritability in single trials (DeLacy *et al.*, 1996a). The BLUP means can be obtained by a REML analysis performed on the genotype-environment cell means, holding genotype and G×E interaction as random effects (while environment is fixed) (Hill and Rosenberger, 1985). Specific models may be applied to unreplicated trials or more complex data structures, e.g. sites and farms (or years) within site (Smith, Cullis and Thomson, 2001; Coe, 2002). BLUP entry means also provide more realistic predictions of yield gains from actually selected entries than do BLUE means.

Breeding for wide or specific adaptation can account for yield stability by selecting entries according to Kataoka's index of reliability (see Section 20.2.7) instead of mean yield over selection environments. This measure is justified by the fact that all G×E effects (including G×L ones) influence the consistency of response and the value for farmers of a variety across the target region or subregion.

20.3.6 Identification of genetic resources, adaptive traits and useful markers

The analysis of adaptation can also produce information on germplasm which, within a given adaptation strategy, is of special interest as parent for crosses or as population for recurrent selection in view of its adaptive response. In general, evidence points to a moderate heritability of adaptation parameters (Becker and Léon, 1988). For example, crosses for wide adaptation could be envisaged between genotypes: (i) possessing high mean yield and the desired adaptive response (as indicated by $b \approx 1$ in joint regression, β value near zero in factorial regression, and genotype PC score near zero in AMMI analysis); or (ii) between pairs of genotypes possessing high mean yield and specific adaptation of contrasting type, such as 'Orchesienne' and 'La Rocca' in Figure 20.4(A). Genotypes may also be classified for adaptive response by pattern analysis (Cooper, DeLacy and Basford, 1996). The analysis of adaptation of genetic resources with contrasting origin may highlight the relationship of the environment of origin to the adaptive response as determined by evolutionary adaptation. Finally, the comparison of different variety types may contribute to decisions of breeding programmes on the genetic structure of novel germplasm (Brancourt-Hulmel, Biarnès-Dumoulin and Denis, 1997).

The analysis of adaptation may also provide preliminary indications on traits contributing to wide or specific adaptation, by correlations of the estimated genotype adaptation parameters with morphophysiological traits (possibly recorded in a subset of test

sites) (Annicchiarico, 2002a). Additional correlations for distinct subregions may contribute to highlight the adaptive traits of local interest. For qualitative traits, or quantitative traits largely definable by just a few contrasting levels (e.g. tall vs. semi-dwarf), the relationship of the trait with adaptive responses can be: (i) inferred visually, by indicating also the plant type in genotype ordination diagrams; or (ii) estimated, by averaging the adaptive responses across genotypes belonging to the same plant type.

The identification of adaptive traits by the above procedures usually needs be confirmed by further results relative to a large genotype sample tested in a few sites (or managed environments) representing different subregions or contrasting environments. The assessment may include a wider set of traits; assess also curvilinear relationships of yield with trait levels; and thoroughly assess the value of single traits or sets of traits as indirect selection criteria. Recent crop simulation models that incorporate gene action may contribute to define adaptive traits by predicting the impact of single traits or trait combinations on genotype adaptive responses to different subregions or contrasting environments (Chapman *et al*., 2002).

Genotype adaptive responses and QTLs for yield may be studied concurrently, to locate molecular markers that could assist the selection for wide or specific adaptation and help locate parent combinations with complementary useful characteristics. QTLs can be accommodated in a factorial regression model by including, in place of the genotype factor, a set of genotypic covariates accounting for the different QTLs (Vargas *et al*., 2006). Also AMMI analysis can be used to map QTLs associated with wide or specific adaptation (Romagosa *et al*., 1996). The information on QTLs and useful markers may derive from experiments performed on just a few sites (or in managed environments) that represent contrasting subregions or environments (e.g. Ribaut *et al*., 2007). Such environments may also be used to compare selection strategies that exploit markers of wide or subregion-specific interest in terms of actual yield gains.

20.4 COPING WITH MICRO-ENVIRONMENTAL VARIATION

Genotypes vary across experiment replications, owing to micro-environmental variation for soil fertility, soil depth or other factors. The portion of this variation that is not controlled by the experimental design produces a special type of G×E interaction (e.g. the genotype × block interaction in a RCB design) that represents the experimental error in the data analysis. Adopting efficient experimental designs and convenient blocking of treatments, and exploring and correcting for the within-block spatial variability, are important tools to minimize this error and increase the accuracy of entry comparisons and, in selection trials, the selection gain. This is particularly important in a participatory programme where most of the trials are conducted in farmers' fields.

The availability of suitable software allows for the ordinary use of efficient experimental designs. For early selection stages, the possibly large number and the limited seed amount of the tested entries may lead to adopting an augmented row-column design with unreplicated entries and replicated controls, such as those proposed by Federer, Nair and Raghavarao (1975) or by Lin and Poushinsky (1983). The latter design, in which most control entries are allocated systematically (at the centre of

subplots, surrounded by eight test entries) while the others are randomly placed to estimate the experimental error, allows for an accurate and flexible adjustment of test entry values as a function of: (i) the row and column effects; or (ii) the covariate represented by the value of systematic controls (which estimates the local yield potential). As a development of Federer, Nair and Raghavarao's (1975) design, a partially replicated design implies two replications for a subset of test entries to improve the estimation of the experimental error (see also Section 20.3.1).

Resolvable incomplete block designs, in which the incomplete blocks can be grouped to form a complete replication of the entries, are preferable to non-resolvable ones because this double-blocking structure allows for better error control and for the possible analysis as a RCB for missing data (Basford *et al*., 1996). To partly overcome the constraints of lattice designs for numbers of tested entries and of plots within incomplete block, Patterson, Williams and Hunter (1978) devised a new class of resolvable designs termed alpha lattices in which, given k plots per incomplete block, the number of entries g may be whatever multiple of k. The efficiency of these designs increases when k approaches the square root of g.

The layout of farmer-managed selection trials depends on the total number of test entries and the plots available per farm. Within these constraints, it should preferably allow for estimating an experimental error on the farm, e.g. by unreplicated trials including a few replicated entries, or on the site and its surroundings, by assigning a complete block to each of few farms or an incomplete block to each of several farms. In less favourable instances or when farm size is small (see also Chapter 9 in this volume), each farm within a subregion may host an incomplete block.

Experimental designs cannot control the effects of environmental variation within complete or incomplete blocks. These effects may be large in stress-prone sites and in trials with many entries. For plots arranged in a rectangular row-column array, the ANOVA residual E_{mn} of the plot located in row m and column n may be modelled spatially as a function of: (i) uni- or bi-directional fertility gradients estimated by polynomial functions of row (R_m) and/or column (C_n) numbers, e.g. a second-degree polynomial response surface; (ii) the covariate X_{mn} represented by the mean value of the ANOVA residuals for the neighbouring plots (which estimates the local yield potential), through a nearest-neighbour (or Papadakis') method: $E_{mn} = b\ X_{mn} + e_{mn}$ (where e_{mn} is the non-modelled residual) (Brownie, Bowman and Burton, 1993). As a third, more complex approach, Cullis and Gleeson (1991) proposed modelling the residuals as a function of the spatial autocorrelation between pairs of neighbouring plots assessed separately along rows and columns. If there is spatial pattern, the variance between residuals of neighbouring plots will be lower than that between residuals of plots far apart, leading to autocorrelation (estimated by a moving average). This two-dimensional separable autoregressive spatial model of first order (AR1 × AR1) can describe many spatial patterns and has frequently proved adequate. Therefore, Gilmour, Cullis and Verbyla (1997) proposed generally fitting this model and then displaying by various diagnostic tools (e.g. the variogram of non-modelled residuals along rows and columns) the possible presence of remaining pattern, to be modelled by additional covariates (e.g. a cyclic row or column effect due

to sowing or harvesting operations) in a REML analysis. The best model is usually selected so as to minimize the Akaike Information Criterion. Trial-specific spatial parameters and other parameters relative to trial design, genotype, environment and G×E effects may all be included into a comprehensive model for estimating BLUP entry means (Smith, Cullis and Thomson, 2001; Smith *et al*., 2002).

Spatial analysis is a trial-specific, iterative exercise that may be relatively time-consuming, especially for complex models or large sets of selection trials. It has tended to reduce the experimental error to a large degree in RCB and to a sizeable degree in lattice designs in extensive studies (Smith *et al*., 2002; Singh *et al*., 2003). However, an assessment of this tool and the two designs in terms of predicted yield gain for wheat selection trials in Australia indicated the sizeable advantage of spatial analysis over the ordinary lattice design analysis in the range of one to five selection trials (test environments), as well as the sizeable advantage of lattice over RCB up to about seven trials (Qiao *et al*., 2004). On the whole, this study highlighted the basic importance of using a lattice design, suggesting modelling spatial variability only in small sets of trials or in trials that exhibit unusually high experimental error.

20.5 COMPUTER SOFTWARE

Nearly all the reported analytical techniques are difficult to apply by breeders without suitable and relatively user-friendly software. IRRISTAT, renamed as CROPSTAT from its sixth version onwards, has been developed by the International Rice Research Institute (IRRI, 2007). It is freely available and has specific modules for plant breeding designs (alpha lattices, augmented row-column design, etc.) and ANOVA, joint regression, AMMI and pattern analysis (outputting most of the relevant graphics). This and other software usually focus on the analysis of G×E effects, and may require some modification to the recommended procedures when analysing G×L effects in trials repeated over time. The use of CROPSTAT in this context has been described by Annicchiarico (2002a) for the main analyses considered in this chapter, including factorial regression analysis (for which no specific module is available). Some recently-implemented tools, such as a REML analysis module usable for estimation of variance components and spatial analysis and a module for generic cluster analysis, have widened or simplified the assistance to data analysis offered by this software.

MATMODEL is software for AMMI and joint regression modelling, which is also available in a free version (Gauch, 2007). It is particularly useful for handling missing data, and its output includes the AMMI-based definition of subregions for genotype targeting. GEBEI is free software for pattern analysis, whose utilities have largely been included into CROPSTAT. It has the unique feature of allowing classification of sites in largely unbalanced data sets according to DeLacy *et al.* (1996b) (contact: Professor K.E. Basford, University of Queensland).

Useful commercial software includes: (i) AGROBASE, allowing one to perform ANOVA, joint regression and AMMI modelling, analysis of unreplicated and replicated designs, and spatial analysis; (ii) INTERA (Decoux and Denis, 1991), with modules for factorial and joint regression and AMMI modelling, ANOVA, and classification of sites and genotypes; (iii) GGE BIPLOT (Yan and Kang, 2003), useful for joint regression and

AMMI modelling, ANOVA, GGE biplot and pattern analysis; and (iv) ASREML (Gilmour *et al*., 2006), powerful for REML and spatial analyses.

GENSTAT, compared with other powerful generic software (e.g. SAS; S-PLUS), includes specific procedures for many analyses (e.g. joint and factorial regression; AMMI; REML; spatial trend) and has a policy of free licensing to institutions of less developed countries. Sets of SAS instructions for several aspects of G×E interaction, REML and spatial analyses are reported in Kang (2003). SAS instructions for specific analyses can also be found in many individual papers.

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CHAPTER 21

Variety release and policy options

Zewdie Bishaw and Anthony J.G. van Gastel

21.1 HISTORICAL DEVELOPMENTS AND CONTEXTS

Empirical evidence shows that for millennia farmers selected plants from their local landraces and saved their own seed for planting. In the 1880s, early attempts in scientific plant breeding began and the first agricultural research stations were established in some European countries (Kåhre, 1990). The history of the organized seed sector is linked to the introduction of new crops and knowledge-based agriculture, including scientific plant breeding, mechanization, commercialization and diversification at various stages of agricultural development (Tripp, 2001; Thomson, 1979). Continued specialization eventually brought significant changes in seed provision, giving birth to an integrated and market-oriented organized seed sector in developed countries (Groosman, 1987).

In many developing countries, however, information on the history of agricultural research and organized seed production prior to 1950s is rather scanty. The introduction of highly productive semi-dwarf wheat and rice cultivars in the late 1960s and 1970s, which triggered what is referred to as the *Green Revolution*, probably served as a stimulus for introducing agricultural research and the establishment of organized seed production throughout the developing countries, particularly for economically important and strategic food crops. From the outset the seed system was inherently service-oriented, with no or limited commercial interests.

21.1.1 Seed system definitions

The entire seed supply of a country comes from different sources, including off-farm from commercial sources such as the public or private sector (formal sector) or farm saved or through local exchange and trading (informal sector). In recent times the concept of seed system has been broadened to include the role of the 'informal' sector in seed provision. Van Amstel *et al*. (1996), apart from providing a comprehensive definition of the seed system, recognize two distinctive, but interacting seed delivery systems: the formal and the informal sectors. A farmer may have adopted a modern variety from the formal sector, but may decide to save seed from their own harvest or exchange through social networks for the next season's planting: seed that is produced informally (Bishaw, 2004).

Formal seed system

The formal seed system is composed of several interrelated components, namely: (i) variety development, evaluation, registration and release; (ii) seed production, processing and storage; (iii) seed marketing and distribution; and (iv) seed quality assurance. It is a highly interdependent chain of operations whose overall performance can be measured by the efficiency of the different linkages in the chain (Pray and Ramasawmi, 1991). In general it is a vertically organized (Louwaars, 2002), large-scale operation, mostly with commercial interests.

Informal seed system

At present, in developing countries, over 80 percent of crops are sown from seed stocks selected and saved by farmers or exchanged and traded locally (Almekinders, Louwaars and de Bruijin, 1994; Alemkinders and Louwaars, 1999). The informal seed system operates at local level (Cromwell, Friis-Hansen and Turner, 1992), and may depend on indigenous knowledge of plant and seed selection, sourcing, retention, management and local diffusion mechanisms (Bishaw, 2004). Apart from farmer or community practices it also includes various local-

level seed production initiatives organized by farmer groups or NGOs, or both, working outside the regulatory regime of the organized seed sector.

21.2 CURRENT PRACTICES IN VARIETY RELEASE

Crop improvement has been an important strategy for the development of the agricultural sector in both developed and developing countries. Modern crop varieties, the results of science-based breeding, are the backbone of the seed industry and indisputably the most critical output of investments in agricultural research. These varieties should be made available to farmers through an efficient, effective and transparent release system to benefit producers and to realize the impacts from investments in plant breeding and variety development. The procedures described below presents the requirements applicable to varieties developed through formal plant breeding by the public and private sectors some of which could be of limited relevance to those emerging from participatory approaches.

21.2.1 What is a variety?

The definitions of variety are many and varied, but the following is probably more practical and concise. According to Carson a '*variety*' is defined as:

> *an agricultural unit created and maintained by man, the first essential being that it should have an individuality which can be reproduced over a number of years, and secondly that it should be distinguishable by inherited morphological or physiological characters from other varieties.*

At present, however, the term variety extends beyond the production field of farmers into expectations of industry and consumers.

21.2.2 What is variety release?

'Variety release' encompasses a broadly interrelated series of activities, from identifying promising lines for further testing to releasing a new variety and making available breeder seed for further multiplication, and the activities may include: (i) identifying promising lines with preferred traits for further evaluation from advanced variety trials; (ii) testing of new promising lines for registration (Distinctness, Uniformity, Stability = DUS) and performance (Value for Cultivation and Use = VCU) by a competent independent authority; (iii) approval of the new varieties for commercial use by a release committee; (iv) inscription of the varieties in the national catalogue; and (v) making available breeder seed of new varieties for further commercial seed production and distribution. Variety release procedure is a collective term that refers to the release type, the attached terms and conditions, the protocols and administrative procedures used in releasing a new variety for seed production and distribution (Delouche and Goma'a, 1999).

21.2.3 Origin of variety release

The beginning of scientific crop improvement enabled skilled breeders and farmer breeders to develop new crop varieties and make available the seed by themselves or through local traders. However, maintaining the identity and purity of the new crop varieties and the proliferation of variety names (Parsons, 1985; Hackleman and Scott, 1990) became a great challenge for the emerging seed industry. Thus, systematic plant breeding brought two important developments in the seed industry: (i) varietal release, i.e. a procedure and criteria for introducing new varieties to commercial seed production; and (ii) varietal certification, i.e. a procedure for

maintaining the identity and purity of new varieties during seed production and supply. According to Tripp (2001), listing varieties based on morphological characteristics and performance was started as early as 1905 (in Germany), whereas seed certification was started in 1888 (in Sweden).

The establishment of the International Crop Improvement Association (now the Association of Official Seed Certifying Agencies (AOSCA; www.aosca.org) in 1919 (Parsons, 1985) and the Organisation for Economic Co-operation and Development (OECD; www.oecd.org) seed schemes in 1958 (Thomson, 1979) were some of the first attempts to standardize variety release and seed certification schemes. These organizations put in place evaluation, registration and release procedures for accepting and listing eligible varieties, and strict generation control to maintain the varietal purity and identity by establishing standardized certification schemes for commercial seed production (OECD, 2007). Likewise, many governments enacted national variety and seed regulations to implement such types of schemes. Despite a long history of organized seed sectors, many developed countries enacted comprehensive variety and seed regulations only fairly recently.

21.2.4 Current procedures and practices

Once new and potential promising lines are identified by agricultural research, it is essential to commercialize and make their seed available to farmers. Variety release requires simultaneous testing of these promising lines for registration (DUS) and performance (VCU) before approval for large-scale seed multiplication and marketing for commercial use. The distinctness (uniformity and stability) of the variety to establish its identity (registration testing) and its commercial value for cultivation (farmers) and use (consumers) (performance testing) are the basis for final reelase.

The ability to discriminate and identify varieties of agricultural and horticultural crops is fundamental in the modern seed trade. Variety description is essential for effective implementation of: (i) variety maintenance (purification); (ii) seed multiplication (roguing); (iii) seed certification (field inspection); (iv) granting intellectual property rights (plant variety protection); and (iv) protection of producers and consumers (seed certification).

The degree to which the breeders are involved and the way release procedures are organized and conducted is described as a compulsory (e.g. European Union) or a voluntary (e.g. United States of America) release system.

Variety registration testing

Variety registration (DUS) testing is a descriptive assessment to establish the identity of the new variety using morphological characters, as well as its sufficient uniformity and stability. DUS testing usually runs for two independent growing seasons or years, where the new variety is compared with a wide range of existing varieties to establish its identity. A detailed DUS testing procedures and crop specific guidelines are available from the International Union for the Protection of New Varieties of Plants (UPOV; http://www.upov.int/en/publications/tg_rom/tg_index.html).

- *Distinctness*: A new variety must be different from existing varieties and must be recognizable to verify its identity and purity during seed production and use. Distinctness refers to a difference from any other variety whose existence is a matter of common knowledge.

- *Uniformity*: It relates to the degree of variability within the variety. The variation observed must normally be of a demonstrable and repeatable order. The variety must be sufficiently uniform within its population so that individual plants could be identified to guarantee constant quality.
- *Stability*: It refers to the capacity of the variety to reproduce itself during seed production without losing its distinctive characters. The genetic make-up of the variety should remain the same during subsequent years of seed production and commercialization.
- *Varietal Identity*: The identity of a new variety is established by examining and describing the morphological characters of growing plants. The purpose of registration testing, whether backed by legislation or not, is the recognition of varietal unit as a unique entity and to establish its identity.

Variety performance testing

Performance (VCU) testing, referred to as 'variety trials', focuses on the merit of the new variety to the end users, i.e. producers and consumers. The test ensures that only varieties that are found better than the existing varieties in one or more agronomic character, such as grain yield or quality, or tolerance to biotic or abiotic stresses, are released for use by farmers. The multi-location and multi-year variety trials are conducted in different agro-ecological zones to identify better performing varieties, which could meet diverse agronomic or consumer requirements or socio-economic conditions. As a result, different agronomic management practices are used and the new varieties are compared with well established commercial varieties. VCU tests usually run for two to three years, where the best performing varieties are eventually recommended for cultivation. In some countries (e.g. Ethiopia) the variety is tested in on-farm verification trials under farmer management practices before the final release.

Variety release

Variety release is a culmination of several interrelated activities, where a decision is taken to approve a new variety for commercial use, based on the results of registration and performance testing. Almost all countries have a variety release procedure in place, whether that is done by an *ad hoc* committee (e.g. the Syrian Arab Republic) or by a legally sanctioned authority (e.g. Turkey). The varieties that meet the requirements for registration and performance are officially released and the owner makes breeder seed available for commercial seed production and marketing. In some countries, however registration testing (DUS) is not yet everywhere part of the requirement for variety release (e.g. Ethiopia).

Variety registers

The new variety, upon approval, will be listed in a variety register to inform the stakeholders, i.e. seed producers, farmers, consumers and the industry. The list could be informative or recommendatory. The register is periodically updated by removing obsolete and entering new varieties that are eligible for commercial seed production at national or provincial levels (e.g. Pakistan). Many countries have a national variety register (e.g. Crop Variety Register in Ethiopia), while OECD has a common variety catalogue (www.oecd.org), which enables the variety to be produced and marketed in all member countries.

Variety protection rights

New crop varieties can be granted breeder's rights in countries with plant variety protection (PVP) laws. DUS testing is part of the requirement, irrespective of the performance or agricultural value of the new variety. However, simple DUS testing alone does not qualify the variety to receive protection. Under the UPOV convention, the variety, apart from being distinct, uniform and stable, must be novel, without prior commercialization and must have an acceptable denomination, before granting the rights for protection.

21.3 RATIONALIZATION OF THE VARIETY RELEASE SYSTEM

At present, there are many policy, regulatory, institutional, organizational and technical constraints affecting the seed industry in many developing countries. The increasing trend towards commercialization of agriculture, the development of private seed industry, the effects of IPRs and the continued decline in public sector agricultural research calls for public-private sector partnership in agriculture research (Morris and Ekasingh, 2002). In the face of changing seed industry, it is imperative for many countries to either reform or to consider revising their policy and regulatory frameworks, as well as technical guidelines and procedures for variety development, evaluation, registration and release. These include rationalizing and developing policy guidelines for variety release systems, enacting variety regulations, review of release procedures, participation of stakeholders, and seeking protection for new varieties. The policy and regulatory reforms must strike a balance between public sector interests, opportunities for promoting private enterprises, and consumer protection.

21.3.1 Policy reforms

It is important that the policy for a variety release system, including the guidelines, processes and procedures, is transparent, equitable, documented and officially sanctioned. Developing flexible and responsive variety development and variety release options are necessary, given the diversity of crops, the level of research conducted on each, and variations in their economic importance, as well as the diversity of seed producers and suppliers.

Public or private plant breeding?

In developed country seed industries, the private sector plays an important role in plant breeding as part of product development strategy. For example, multinational seed companies tend to reduce transaction costs through vertical integration of the research–seed production–seed distribution continuum to recoup their investments (Morris, 2002). In contrast, in many developing countries, historically the public agricultural research sector predominates and has sole responsibility in setting the national research and crop improvement strategies and priorities. Past government policies always tended to support public over private sector plant breeding and may restrict the development of both domestic and foreign private sector operations (Tripp and Louwaars, 1997). Particularly for crops considered strategic for a country, there are general protectionist tendencies for public sector plant breeding and varieties (Bishaw, Manners and van Gastel, 1997). It is important for governments to encourage public-private collaboration and partnership in agricultural research and plant breeding (Morris and Ekasingh, 2002) to exploit synergy and make available a wider choice of varieties to different sectors of the farming community.

Unrestricted or exclusive variety release procedures?

Commercialization of public-bred varieties can follow unrestricted (open) or exclusive releases. 'Open' releases do not provide adequate incentives for investments in promoting varieties because the participation of other seed companies diffuses the benefits. In exclusive release, however, one or a limited number of seed producers get access to varieties under specific terms of negotiated fees or royalties, and is the most common procedure in countries where PVP exists. Delouche and Goma'a (1999) suggested different variety release options for public-bred varieties. Some of these and other options are presented and discussed below.

- *Open and unrestricted release without royalties.* To date, many participatory plant breeding and alternative seed delivery systems are operating at the local level, aimed at improving farmer access to varieties and seeds in less favourable environments and remote areas. Such initiatives focus on small-scale farmers growing minor crops, which are of great importance for their livelihoods and food security, but with limited commercial value, and so attract investment from neither the public sector nor the private sector. To ensure local-level seed initiatives, small-scale seed enterprises should have open and unrestricted access to public varieties. This procedure is most suitable for minor crops with little commercial potential due to limited area planted and a very slow rate of variety replacement, or for varieties emerging from participatory approaches.
- *Open and restricted release with royalties.* Under these conditions all qualified seed producers can get access to Breeder seed of new varieties, but also pay royalties proportionate to commercial seed sales. It could probably continue to be a common variety release procedure for major self-pollinating crops (e.g. wheat) until there is tangible progress in private sector participation and provision of PVP. The public research organizations may be interested in generating additional resources to augment declining funding and support their breeding programme by charging royalties or selling variety rights. In some countries, in the absence of PVP, royalties are paid for public-bred varieties (e.g. Egypt).
- *Exclusive release with royalties.* Exclusive releases should be considered, especially when PVP becomes available and could also extend to some major self-pollinated crops (e.g. wheat). The exclusive releases can be made to a single company, group of companies, associations or cooperatives. Experiences from developed countries show that exclusive release of a variety with broad adaptability justifies investments in the promotion and market development strategies that are critical for gaining rapid and wide variety adoption by farmers. It is also possible to broaden the scope of variety release by transferring the proprietary rights to other private seed companies for commercialization purposes.

Compulsory or voluntary variety releases?

Previously, variety release schemes developed independently without prior knowledge of what happened in other countries, but later improved and expanded to meet the challenges in plant breeding, seed production and farmers' interests (Parsons, 1985; Hackleman and Scott, 1990).

EU member countries follow a compulsory variety release system where both registration and performance testing are

handled by an independent agency. In many developing countries, following the examples of EU, the governments strictly regulate the introduction of new varieties, prohibiting seed production and marketing until the variety is tested by a government agency and approved by the release committee (Gisselquist and Sirvastava, 1997). The variety should meet both DUS and VCU criteria for release. The problem is exacerbated by lack of a competent agency to implement an impartial release system.

In the United States of America, both variety registration and performance testing is voluntary, where, based on the available data, the responsibility and decision lies with the plant breeder to release the variety for commercial use. In India and the Philippines, a mixed mandatory and voluntary variety release system operates, based on the crops (major or minor) or the enterprises (public or private). Voluntary variety release systems favour competition, lowers the cost, allows easy entry of new seed companies, and offers more choices for farmers. Breeders ensure that the variety meets the requirements of the producers and users for commercial success.

To date, more and more collaboration can be seen between breeders and the variety release agency, including countries that have adopted the compulsory system. Therefore, governments have to adopt policy changes and encourage voluntary variety registration and performance testing, where greater responsibility is given to the breeders and the industry.

Single or multi-country variety lists?

In many developing countries, the National Agricultural Research Systems (NARS) receive almost similar sets of breeding lines of major food crops, supplied through a network of International Agricultural Research Centers (IARCs). Despite similarities in agro-ecology, farming system and germplasm, there is no mechanism for sharing data in making decisions concerning variety release, even between neighbouring countries, with the result that often the same breeding line is released under different names across countries. Each country organizes its own independent mandatory registration and performance testing for variety release, leading to single-country variety lists. Although the EU has mandatory variety registration and performance testing, any variety that is registered in the common catalogue can be marketed freely in all member countries. It is important that countries accept varieties that are listed in other neighbouring countries with similar agro-ecology without going through repeated lengthy release system, i.e. that there be a multi-country variety list. In Turkey, foreign-registered varieties from member countries of EU, OECD and UPOV are exempt from DUS testing and are accepted as part of the requirement for variety release.

In 2005, apart from providing breeding materials through international nurseries, ICARDA initiated a regional testing scheme where all released wheat varieties from the Central Asia and Caucasus are tested for adaptation across the region. It is highly desirable to encourage countries to move from mandatory to voluntary, and from single- to multi-country lists, in variety release (Gisselquist, 1997) to increase the choice and movement of varieties and to harness the impact of plant breeding research at national, regional or global levels.

Access to public sector varieties

National agricultural research systems serve as direct conduits of public-bred varieties to

farmers. They directly channel Breeder seed of new varieties to public companies for further seed production and distribution. The access of the private sector to public bred varieties remains a major stumbling block in many developing countries. This is particularly important for domestic, small, private seed companies and small-scale seed enterprises operating at local level serving farmers in less favourable areas, but which rely on varieties from public-sector sources. Such seed companies have neither the resources nor the technical capacity to engage in plant breeding programmes. It is essential to have a transparent and equitable mechanism to guarantee access to public-bred varieties, as discussed under the variety release options.

21.3.2 Regulatory reforms

Variety development, evaluation and release are closely interconnected, and it would be difficult to draw distinctions between the regulatory frameworks that govern these as two separate activities. Accordingly, variety regulatory frameworks are the rules and regulations associated with variety development, testing, registration and release (Tripp, 1997). In effect, it includes the procedures and practices that guide the conduct of plant breeding; the rules governing the official release of new varieties; and restrictions on the type of varieties that may be offered for sale.

Tripp *et al* (1997) described the key features and limitations of variety and seed regulations, and their introduction to developing countries. Most of these regulations are modelled upon and influenced by past historical relationships and source of donor support to national seed programme development. They are at times excessively strict and inflexible, limiting the range of varieties and seeds available to farmers. Tripp (1995) argues that regulatory reforms must be seen as a continuing process, and must be sufficiently flexible to respond to and promote the evolution and diversification of the national seed sector in developing countries.

In general, the majority of developing countries lack well established variety release protocols and procedures in place. The level of regulation is commonly not in line with the level of institutional development of the country, leading to incomplete implementation and insufficient transparency. This creates a serious lack of credibility and inconsistent application of these regulations by the authorities. Tripp *et al.* (1997) identified four key constraints that need to be addressed in regulatory reform, namely: (i) the efficiency of operation; (ii) application of objective and relevant standards; (iii) participation of stakeholders; and (iv) transparency in managing registration and performance testing for variety release.

Harmonization of variety regulations

The regulatory requirements governing registration and performance testing are critical elements in variety release mechanisms. In the past, where these regulations existed, they were prepared and implemented within their specific national context. Some countries have comprehensive variety regulations, whereas others still have no or outdated legislation, which consequently do not meet the needs of a modern seed industry. With globalization, these inflexible regulations are a serious impediment to movement of varieties across national boundaries, thus severely limiting opportunities in regional and global seed trade. Harmonized variety regulations (e.g. East African Community) would increase the choice and movement of

varieties and seeds across national borders and stimulate regional seed trade. Given the diversity of national seed systems and the globalization of the seed trade, it would be appropriate to develop a variety regulation and release procedure that is both flexible nationally and harmonized regionally.

Introducing plant variety protection

Plant breeding is a long-term process with substantial financial investments. To encourage investment in plant breeding it is important to have legal protection for companies to recuperate their investments. Lack of PVP is often considered a major constraint for the limited or non-engagement of multinational and domestic private seed companies in seed markets of developing countries. As discussed elsewhere in this chapter, however, in practice things seem to be changing, where public bred varieties are auctioned (e.g. Morocco) or public sector breeding programs enter into bilateral agreements on royalty payments with seed companies in the absence of PVP system (e.g. Egypt).

It is anticipated that strengthening PVP would encourage private sector investment in plant breeding and diversification of the seed sector, making more varieties available to farmers. For example, within Central and West Asia and North Africa region some countries are UPOV members (Azerbaijan, Jordan, Kyrgyzstan, Morocco, Tunisia, Turkey, Uzbekistan) whereas others (Algeria, Egypt, Pakistan and Tajikistan) are preparing laws to join the Union. Some countries (e.g. Ethiopia, Yemen) have legal instruments for variety protection that may satisfy TRIPs requirements, though not in conformity with UPOV convention. Although the expansion of the IPR concept has generally appeared to strengthen the incentive for private-sector investment, there is still lack of conclusive evidence on its impact on the commercial plant breeding industry (Morris and Ekasingh, 2002), on diversity of varieties in farmers' fields (Fischer and Byerlee, 2002), and as a precondition for the development of the private sector (Louwaars *et al*., 2005).

Introducing biosafety laws

The Cartagena Protocol on Biosafety (CBD, 2000) sets out a comprehensive regulatory system for ensuring the safe transfer, handling and use of living modified material (LMOs) resulting from biotechnology and subject to transboundary movement. The protocol is envisaged to encourage innovation, development and capacity building in relation to biotechnology, while also achieving the goals of conservation, sustainable agriculture and equitable sharing of the technological benefits. However, the introduction of transgenic crops forced countries to develop biosafety regulations that make the release of 'biotech crops', both for testing and for commercial use, dependent on extensive release procedures. Should the use of such varieties become more widespread, there might be stricter and comprehensive release procedures under the pretext of biosafety laws.

21.3.3 Technical reforms

Apart from policy and regulatory frameworks, there is a need for technical reforms responding to the needs of diverse stakeholders.

Availability of farmer-preferred varieties

Formal plant breeding received considerable criticism for not paying sufficient attention to the crops and conditions of farmers in less favourable areas. For example, yield performance is given considerable weight compared with farmer-preferred traits such

as cooking quality, taste, marketability and storability under traditional farming systems. Moreover, the strategies tend to favour wide adaptability and selection of material under favourable crop management, where both the environment and the trial management are unrepresentative of actual farmer circumstances. In the end, only a few 'average best' varieties (Alemkinders and Louwaars, 1999) become available from public plant breeding, which too often will subsequently be produced and distributed by inefficient public seed companies, limiting the choice of varieties and availability of seed to farmers.

In contrast, farmers in diverse, complex, dry and risky environments are interested in varieties with a broad genetic base with the capacity for individual and population buffering in stress environments, such as heterogeneous populations. Consequently, several varieties with specific adaptation are preferred over a few varieties with wide adaptation. Although selection is more effective in the target environment, marginal environments are inadequately represented in national breeding programmes, or even ignored.

In many developing countries, the responsibility for variety development rests with public NARS. Therefore, the NARS plant breeding strategy, protocols and procedures have greater influence on the type and number of varieties available for release. There are serious concerns regarding efficiency and effectiveness in the variety development process, the criteria used in evaluating breeding materials, the degree of stakeholder involvement and the transparency of the system (Witcombe and Virk, 1997).

Criteria for variety release testing

In many developing countries, registration and performance testing for varietal release have appeared to be a bottleneck for the flow of modern crop varieties from agricultural research to farmers. The major criticism of the variety release system is the stringent requirement and application of detailed DUS and narrowly defined VCU criteria implemented by public-sector agencies. For varieties produced by conventional plant breeding, it is important to develop clear, simple and flexible registration and performance testing systems based on criteria developed by all stakeholders. It is important that for variety registration some key descriptors are identified and used to help seed producers and certification agencies recognize varieties, instead of detailed examination and recording using a large number of morphological characteristics. Although conventional regulation might intend to abandon variety registration, the introduction of variety protection laws does require a detailed registration of the protected entity. Tripp *et al* (1997) suggested that requirements for conventional registration and for granting PVP be treated differently and handled by separate agencies to minimize complications. Similarly, they suggested that the performance testing should reflect the circumstances and preferences of farmers, where suitability instead of superior yield is used as the criterion for variety release.

Prolonged testing in variety release

Developing a new crop variety to enter testing for release may require more than 10 years. Thereafter, the variety must also pass through series of preliminary yield trials and meet the requirements for registration and performance testing for official release. It may take another two to six years before the variety is finally approved for release. Seed production can only be initiated following the official release, meaning that it might require an additional five

or more years for a sufficient quantity of commercial seed to reach the farmers. In general, there is considerable delay and cost in the process from variety development through to its release and availability of seed for commercial use, which is a very lengthy process. The variety development and release process may take up to 15 years (e.g. Uzbekistan) from the initial crossing nursery to the end of official state variety testing for variety release.

In the public sector, variety development, variety release and seed production and marketing are conducted by different institutions, which are not properly linked and this may exacerbate the problem and prolong the period compared with the private sector, where these activities are integrated. Seed production and marketing start only after the official release, and there is no inbuilt mechanism for pre-release seed multiplication of public-bred varieties to expedite the availability of seeds, with a few exceptions (e.g. Ethiopia, Uzbekistan and Zambia).

Harmonizing testing and release procedures

The UPOV protocols are becoming internationally accepted standards in DUS testing for variety description, registration and protection. Under the UPOV convention, to maximize use of available information and minimize the time for examination, there is cooperation among countries or authorities, where some institutions have been identified and specialized in DUS testing for specific crops. The EU is a good example of a regionally harmonized release system, where varieties released in one country are acceptable in all member countries. Such an approach provides great opportunity for developing testing protocols and sharing data, as well as establishing flexible and harmonized variety release systems in regional or international contexts.

Harmonization of release procedures are naturally an extension of harmonized variety regulation. As discussed earlier under regulatory reforms, collaboration among countries and sharing of data could enable much quicker decisions on variety release. Using data from other countries to reduce the number of years or to waive requirements based on data submitted from tests carried out elsewhere is critical. In recent years, for example, efforts have been underway to harmonize variety release procedures to integrate national seed systems to attract foreign investment and promote regional trade as part of harmonized variety and seed regulation (e.g. Community of Andean Nations). Regional harmonization of technical aspects of variety release systems may reduce cost, save time, encourage private investment, increase choice of varieties and benefit farmers.

Managing registration and performance testing

In principle, testing for variety registration and performance should be managed by an independent and impartial agency established for the purpose. In reality, such agencies vary from country to country, and the responsibility may be vested in a single agency or two different institutions. In some cases it is possible to make decisions based on tests carried out by the breeder, but at the discretion of the agency. For example, in Ethiopia, Algeria and Jordan, for release their National Variety Release Committees depend on performance testing data supplied by the breeder and on-farm verification trials conducted by the breeder, but reviewed by a special technical committee.

In many developing countries, however, there is lack of an impartial authority

responsible for implementation of a variety release system and responsibility rests with the national public agricultural research organizations, which also have the responsibility for plant breeding. In general, the research organizations may have limited infrastructure and financial resources, coupled with possible professional bias, thus precluding operating an independent, effective and efficient variety release system unless some level of impartiality is instituted within its variety evaluation system (Morris and Ekasingh, 2002).

Tripp *et al.* (1997) suggested that it is most important for the agency to perform its task with greater efficiency and expediency by using appropriate criteria in a very transparent and participatory approach. The participation of wide range of stakeholders in the process, particularly the private sector, farmers, development agencies and NGOs, increases the transparency and accountability of the variety release system. It is envisaged that variety release systems accommodate both public- and private-sector-bred varieties in an equitable manner.

There are also suggestions to create linkages between variety registration and performance testing, with demonstration and popularization of varieties to create farmer awareness of the merits of new varieties before final release. In the private sector, variety development, seed production and marketing are integrated because commercial success is dependent on the efficiency of the system. They conduct extensive testing of new varieties early on with on-farm demonstration in farmers' fields as part of their product promotion and market development strategy, with the immediate effect of entering commercialization upon release (Ansaldo and Riley, 1997).

The most important criticisms leveled against variety release committees is their lack of transparency and representation of all seed sector stakeholders. Most often the committee is dominated by plant breeders and public-sector officials, and excludes representation from the private sector and farmers. For example, in Turkey, of 12 members of the Variety Release and Registration Committee, only two represent the private sector and one the farmers, while in Ethiopia, all 10 members of the National Variety Release Committee are drawn from public-sector institutions. Apart from being unrepresentative, variety release committees are quite often marred by professional biases, being dominated by breeders, and meet infrequently, so decisions are not timely. Experiences from many developing countries show that most of the committees have no legal backing and run on an *ad hoc* basis, where the decisions carry less weight in implementing an effective variety release system.

21.4 PARTICIPATORY PLANT BREEDING AND VARIETY RELEASE

In parallel to recognition of the informal sector in seed supply (Almekinders, Louwaars and de Bruijin, 1994), there is also growing interest in farmer participatory approaches, for example in genetic resource conservation (e.g. Ethiopia, see Worede, 1992) and in plant breeding (e.g. Syria, see Ceccarelli *et al.,* 2000). The products of participatory approaches, however, must eventually reach and benefit a sufficiently large group of farmers in order to justify the investment in crop improvement.

Farmers' perceptions and varietal choices

Louwaars (1995) indicates that farmers' varietal choice is influenced by ecological (adaptation), economic (marketing, consumption) and cultural (local use) factors. The perception and preferences of varieties

is somewhat different between commercial and subsistence farmers. The former is more likely to prefer varieties with higher yield and productivity, whereas the latter may consider diverse varieties with more stable yield and multiple end uses. In commercial agriculture, farmers are more likely to increase production and productivity by intensifying agriculture through use of purchased inputs like fertilizers, pesticides, etc., to maximize profitability. Moreover, mechanization, intensification and commercialization of agriculture require uniform varieties for farm operation, industrial processing and consumer requirements. Therefore, in situations where farmers are connected to markets, the potential yield, industrial quality and marketing are the driving forces in varietal choices for production.

In contrast, subsistence farmers practice complex patterns of farming, which may involve the cultivation of many crops, with the primary objective of meeting household food security while still having some marketable surplus, if possible, to meet additional expenditures. The main effort is to maximize the use of land and available resources to minimize the risks associated with farming, through diversification of crops, cultivars, farming and off-farm activities in an attempt to stabilize their income. Small-scale farmers' perception of varieties is different from that of many plant breeders. Apart from yield, factors like grain quality, storability, suitability for intercropping and the use and value of crop residues may all influence their decisions about variety adoption (Haugerud and Collinson, 1990). Small-scale farmers perceive local landraces to be more adaptable to their agro-ecology, give stable yield, perform better under low soil fertility or low inputs, have good grain quality and are suitable for preparation of traditional foods (Bishaw, 2004).

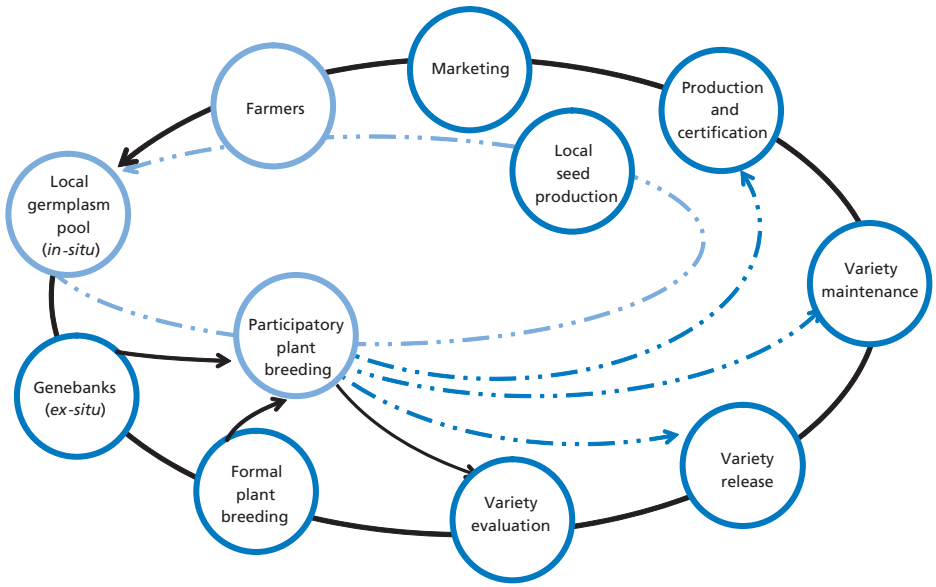
Participatory plant breeding

In many developing countries, conventional (formal) plant breeding (CPB) has shown spectacular progress in developing new crop varieties for uniform and favourable areas where the formal sector managed to produce and market seeds to farmers. However, as the environment becomes complex, dry and risky, there is a clear challenge to breed new varieties to meet farmers' preferences and that are adapted to diverse environmental conditions. Weak rural infrastructure and poor socio-economic conditions further exacerbate these problems.

The limited success of CPB in meeting the need of smallholder subsistence farmers in less favourable environments of the developing world led to the emergence of participatory approaches, focusing on farmer preferences and involvement to encourage rapid adoption and diffusion of new varieties. This could be achieved by bringing the selection process much closer to the farmers, in terms of both the selection environment and their participation in improving the effectiveness and impact of agricultural research. A number of authors described examples of participatory approaches, for example in Rwanda (Sperling, Loevinsohn and Ntabomvura, 1993), India (Joshi and Witcombe, 1996), Nepal (Staphit *et al.*, 1996), Syria (Ceccarelli *et al.*, 2000) and Ethiopia (Belay *et al.*, 2006), and for a wide range of commercial and indigenous crops, including beans, rice, barley, tef, maize, sorghum and pearl millet. The extent of farmer involvement ranges from selecting among nearly finished varieties (participatory variety selection, PVS) to participation in selection on research stations, or to handling segregating populations on farmers' fields (participatory plant breeding, PPB).

The role of participatory approaches in

FIGURE 21.1
Linkages between participatory plant breeding and seed supply



Source: adapted from Bishaw and Turner, 2008.

increasing diversity at farm level, shortening the breeding cycle, identifying well-adapted and acceptable varieties, quicker availability of varieties and seeds, empowering the farmers and lowering overall breeding programme costs have been discussed by several authors (e.g. Staphit *et al.*, 1996; Witcombe *et al.*, 1996, 1999; Mangione, Ceccarelli and Grando, 2006; Ceccarelli and Grando, 2007). Bishaw and Turner (2008) discussed the potential linkages between PPB and seed supply systems to exploit farmers' knowledge in crop improvement and ensure rapid adoption and diffusion of varieties (Figure 21.1). They advocated national policies that recognize the role of PPB and support strategies to release, produce and market varieties developed through these approaches. They also noted critical issues to be addressed for the PPB approach to function properly such as the need for maintaining identity and integrity of participatory varieties, applying flexible variety release procedures, and establishing alternative seed delivery systems. Some of these options are presented and discussed in more depth below.

Institutionalizing participatory plant breeding

Participatory approaches are evolving and still lack clearly defined procedures compared with long-established formal breeding programmes. At least two major forms of participatory approaches have been recognized: PVS and PPB, the latter with many variant forms (breeder/farmer-led PPB, decentralized PPB, highly-client-oriented plant breeding, etc.) and some differences in methodological approaches, type of breeding materials, and stage and degree of involvement by farmers.

Some successes have been reported with participatory approaches, including PVS and PPB in recent years. However, PVS appears less problematic as it deals with already released or nearly finished varieties to derive farmer's varietal choices. At the same time, an attempt to institutionalize PPB in NARS breeding programmes in its own right is still under debate and its future remains uncertain. Were PPB officially recognized and institutionalized, the issue of variety release and its commercialization would have long been resolved at the national level. Therefore, outcomes from PPB need to be documented and its impacts demonstrated to influence national polices to overcome the hurdle.

Maintaining varietal identity and integrity

There are two key factors for adoption and diffusion of a variety: (i) genetic integrity (the inherent capacity of the variety to reproduce itself during seed multiplication); and (ii) varietal identity (its unique distinguishing characteristics established during its development). CPB generates defined outputs (cultivated varieties) with the responsibility for maintaining the variety (identity and integrity) vested in the breeder, or a designated agent, and ensuring a continuing source of pure material as long as it remains in commercial seed production. This system of variety management is absolutely critical in formal systems, since it provides a secure point of reference and, by limiting the number of generations, it also reduces the risk of contamination.

Similarly, it is therefore highly desirable that the identity and integrity of PPB varieties are systematically maintained and made available to more farmers. To achieve this, responsibility should be vested either in the formal sector, in an 'individual farmer-breeder', or more likely, in farmer groups established to produce and market the seed. This provides a basis for some continuity of pure seed supply and to maintain the identity of the material. In the absence of such arrangement, the purity and identity of the variety may dilute and diffuse over years.

Flexibility in varietal release

The disadvantages of formal variety release procedures are discussed elsewhere. However, in PVS, a limited choice of 'finished' or 'nearly finished' varieties bred through conventional or other means are exposed to numerous groups of farmers across villages in widely dispersed geographical areas for farmers to select according to their preferences. In reality, PVS is closer to conventional breeding as it involves farmers only towards the end of the selection programme. Therefore, PVS presents less challenge compared with PPB in variety release systems, particularly if the varieties used are from conventional plant breeding programmes.

In PPB, a few representative farmers are involved in selecting varieties from large segregating populations. It is believed that the PPB approach gives greater opportunity because of wide dispersion of sites that reflect the actual environments of crop production. Consequently, PPB should encourage the use of more adapted material, and development of many varieties with specific adaptation, particularly to less favourable environments. This may increase farmer choice, but it may create challenges for the formal variety release system, and ultimately for seed production as well.

Varieties developed through PPB do not always meet the stringent DUS and VCU criteria because they may lack sufficient uniformity and might not always perform well across the majority of test sites compared

with varieties from conventional plant breeding. Applying identical testing criteria would be unrealistic, and alternative variety release procedures must be considered. The criteria for registration and performance should be flexible and accommodate less uniformity within a variety and allow a wide range of varieties with specific adaptation to increase the choice of niche varieties available to farmers. Possible scenarios for release of materials from PPB are considerd below (Bishaw and Turner, 2008).

Linking to formal plant breeding

Conventional plant breeding exploits indigenous knowledge by involving farmers at different stages in the selection process. The materials identified or selected by farmers can be further refined and the varieties ultimately evaluated and released through the official process and the seed become available through the formal sector. Sthapit, Joshi and Witcombe (1996) described where PPB products entered national trials using scientist-led breeding schemes run in parallel with those of the farmers, with the main purpose being to purify the variety and select for uniformity to meet criteria for formal release. Belay *et al*. (2008) demonstrated where both conventional and participatory approaches were used in a complementary mode for official release of a variety in Ethiopia.

Linking to formal variety release

PPB products identified by farmers can directly enter official variety release and registration trials, but they may encounter difficulties in terms of either uniformity or performance for reasons already explained. It is suggested that release committees accept PPB data on farmer perceptions and demand for seed rather than yield data from scientist-managed trials (Witcombe *et al.*, 1996). Ceccarelli and Grando (2007) outlined the PPB model for barley, where early generation materials (farmers involved from F_3 bulk) go through four cycles of selection, when farmers are involved in selecting and testing the materials during the subsequent years. Farmer-selected varieties in large-scale trials (fourth cycle) are considered adopted and should be released. Alternatively, they suggested that testing pure line (pedigree) selection from selected bulks can be conducted on-station and released in situations where there are stringent variety release requirements.

For PPB varieties, any detailed examinations for VCU appeared to be redundant since farmers are already part of the selection process and identified those meeting their preferences. Some countries also release varieties purely based on performance testing where DUS requirement would not be problematic if farmers criteria are accepted (e.g. Ethiopia). The alternative approach is to release PPB products through a separate registration system established to accommodate these varieties, or even make an outright decision to release them without testing (e.g. Jordan). However, even if the DUS criteria are relaxed, some level of description is essential to identify the variety for purposes of seed production and marketing through formal channels.

Linking to formal seed supply

PPB varieties could be exempted from release systems and directly enter seed production. The formal sector may take direct responsibility for large-scale seed multiplication and marketing of PPB varieties identified by farmers. Given the fact that PPB varieties may have a larger recommendation domain beyond the initial testing sites, it is suggested that large-scale seed production and distribution and external intervention

be used as a strategy to accelerate diffusion (Joshi, Sthapit and Witcombe, 2001).

Alternatively, the formal sector could limit itself to variety maintenance and Breeder seed production, in order to ensure small but secure supplies to local seed producers. Virk *et al*. (2003) emphasized the importance of formal sector (research, universities, department of agriculture), NGOs and self-help groups, supported by government, to ensure seed production and dissemination once farmer-preferred varieties have been identified through a participatory approach. Ceccarelli and Grando (2007) advocate the need for linking PPB varieties to formal and informal channels to ensure adoption and realize impact.

Linking to local seed supply

At present there is limited information on scaling up seed production to diffuse PPB varieties. Despite apparent strengths, local seed systems may not adequately meet requirements for wider distribution of PPB varieties unless they are properly strengthened and linked. Almekinders, Thiele and Danial (2007) consider that there is a tendency to overestimate the role of informal farmer-to-farmer seed exchange as a diffusion mechanism for PPB varieties. In India, a follow-up study for a rice variety identified by PVS showed seed diffusion from farms to relatives or friends in adjacent or nearby villages typically over distances of less than 10 km, despite project intervention in providing seed through village seed pools, seed merchants and NGOs (Witcombe *et al.,* 1999). Some institutionalization of local seed systems is, however, necessary by involving, for example, existing community groups, farmer's cooperatives or associations, local traders and entrepreneurs, NGOs, extension services or rural development agencies, and linking them to the formal sector. For local initiatives to succeed they must address the key issues of financial viability and sustainability without external support (Bishaw and van Gastel, 2008).

Protecting PPB varieties

In the last decade or so, access to genetic resources and protection of plant varieties has become an important part of an increasingly intense debate in formulating policy and regulatory framework at national and international levels (see also Chapters 23 and 24 in this volume). Chapter 23 argues for Farmers' Rights (FRs) under International Treaty, whereas Chapter 24 proposes Plant Breeders' Rights (PBRs) under national IPR laws. In reality there is no contradiction between the two as they address two separate issues. However, quite often there is confusion, mixing farmers' rights with breeders' rights. In Ethiopia, the government has enacted two separate regulations for plant breeders' rights and community farmers' rights,

There are many forms of IPR protection for plant varieties through biological (e.g. hybrids) or legal control including trade secrets, contracts and licenses, patents, and PVP laws. Among these patents for asexually propagated materials (since the 1930s) and latterly for genes, gene combinations and biotechnology products, while PVP laws for plant varieties have been long established as IPR protection systems in the field of agriculture.

First, in conventional plant breeding, describing a variety using morphological characters and establishing its identity is a prerequisite both for release and for protection. For example, under the UPOV convention, granting PVP is based on DUS and novelty of the variety. Theoretically, if PPB varieties meet these criteria it is

assumed they could be granted immediate protection under national PVP law. At the same time, it is argued that PPB varieties may not meet the stringent requirements of the formal seed system and should be hence given special treatment. Could protection rights therefore be given for a variety whose identity is not clearly established? Is it possible to enforce protection in case of infringements of rights?

Second, the fundamental purpose of PVP is to enforce PBRs, which is a private and exclusive ownership right over new varieties, and enforced by breeders to recuperate their investments. Technically, PPB varieties are products of collaboration among different stakeholders, including farmers, communities and breeders. Who is the owner of PPB varieties: the individual farmers, their communities, the collaborating breeders or a 'collective' ownership? Who are the ultimate users or beneficiaries of PPB varieties? Does the legal protection promote or hinder wider use of PPB varieties?

Third, at present, neither FRs nor PBRs provide sufficient regulatory framework to protect PPB varieties, because of complex legal and technical issues. According to the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA; FAO, 2009), FRs are clearly defined irrevocable rights arising from the past, present and future contributions of farmers in conserving, improving and making available plant genetic resources and the opportunity for access and benefit sharing from their use by a third party. At the same time, PBRs are about private rights given on a product whose identity is clearly established and for specific period of time. Who is the source of breeding materials for PPB varieties? Does the scope of FRs under the International Treaty meet the criteria of protecting PPB varieties?

Fourth, the purpose of PPB is to circumvent formal sector constraints in developing and making available farmer-preferred varieties, and their wider adoption and diffusion. It should avoid as much as possible the legalistic and bureaucratic ramifications that might undermine its novel approaches. What is the purpose of protecting PPB varieties? Should PPB varieties be considered a public good for the entire farming community? Is the protection meant to address the public good nature of these varieties?

Does simply invoking FRs as a means to protect PPB varieties necessarily serve the interest of farmers? It is therefore advisable to further elaborate the many uncertainties and unanswered questions surrounding the protection of PPB varieties and develop a working mechanism acceptable to all parties. This will do justice in rationalizing an already burgeoning and complicated legal arena in agriculture. Ultimately, one must acknowledge that countries have the right to design IPR regimes that are compatible with their own agricultural development and serve the interests of their farmers.

21.5 CONCLUSIONS

According to Srivastava (1997), there are profound structural changes and emerging trends in the seed industry, including globalization of agricultural research, shifting to private-sector plant breeding, increased investment in biotechnology, liberalization of seed trade, emergence of private seed companies, entry of multinational seed companies, greater attention to the informal sector, and debate of regulatory and trade agreements on IPR and biodiversity. These changes call for establishing an effective, efficient and transparent variety release system to serve the needs of diverse economies.

At present, however, many countries in both developed and developing countries require comprehensive and mandatory tests for registration and performance testing for new varieties to be released for commercial seed production and use by farming communities. Despite similarities in agro-ecology, farming systems and germplasm there is lack of coordination, collaboration and cooperation among developing countries towards streamlining a common and harmonized variety release system. Moreover, each country has its own variety release system in place. This could be a lengthy process that might be repeated in many separate countries, making it very costly and also leading to serious delays limiting the choice of varieties available to farmers. Countries could accelerate the flow of new varieties to farmers by moving from compulsory to voluntary registration and from single to multi-country lists by harmonization of the system at supra- or sub-regional levels. It is high time to make a critical analysis of policy, regulatory, technical, institutional and organizational constraints and develop a responsive variety release system at both national and regional levels.

Varieties developed through participatory approaches pose new challenges and do not always meet the traditional stringent DUS and VCU criteria because they may lack sufficient uniformity and not always perform well across the majority of test sites compared with varieties from conventional plant breeding. Applying the same testing criteria would be unrealistic and alternative variety release procedures must be considered. The criteria for registration and performance should consider farmer preferences and be flexible and accommodate less uniform varieties, and also a wide range of varieties with specific adaptation to increase the choice of niche varieties available to farmers.

In an era of liberalization and globalization, it is important for national governments to take the lead in providing an enabling policy and regulatory environment to support the development of a competitive and pluralistic seed industry that meets the varietal requirements of the farming communities, given the diversity of seed suppliers, farmers, crops and farming systems.

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CHAPTER 22

Participatory seed diffusion: experiences from the field

Humberto Ríos Labrada

22.1 CONVENTIONAL SEED PRODUCTION SYSTEMS IN CUBA

During the golden years of the eastern Socialist countries, a centralized plant-breeding model was a standard component of the high-input agriculture practised in Cuba, and particularly for the country's cash crops (Begemman, Oetmann and Esquivel, 2000). Foreign varieties, hybrids, landraces and varieties obtained by mutation were the principal sources of genetic variation used for varietal development in Cuban plant breeding programmes (Ríos, 1999). At the end of the 10–12 year period typically spent in varietal development for a specific crop, the breeding programmes usually released only one or two varieties for the entire country, therefore assuming a geographically wide adaptation. Wide geographical adaptation characteristics were encouraged by policy-makers, with most Cuban governmental organizations providing incentives to scientists involved in releasing a variety for use over a large area.

Ambitious plant breeding programmes were developed in the 1980s for sugar cane, roots and tubers, rice, tobacco, coffee, horticultural crops, pastures, grains, fibres and some fruit trees, undertaken by fifteen research institutes and their corresponding networks of experimental stations that spread over the island (Begemman, Oetmann and Esquivel, 2000).

As a part of the varietal release process, each new variety had to pass through a series of steps. The research institutes sent their results to the Scientific Forum (*Consejo Cientifico*) at the national level. This Forum checked their scientific validity and, if approved, they sent them on to an Expert Group (*Grupo expertos*), which consisted of researchers, teachers and production directors. If this group approved the results, they were then sent to the Vice-Minister of Mixed Crops (*Vice-Ministro Cultivos Varios*). This Minister would send the results to the provincial delegations, which would incorporate them into their production plans, so that producers were obliged to adopt them. This procedure took a top-down approach without consulting the producers. Some researchers did visit farms, but still the research agenda came from the decisions of the researchers (Trinks and Miedema, 1999).

Some plant materials collected in Cuba with useful characteristics, such as disease resistance, short growing cycles and good food qualities, were not used by the formal plant breeding sector due to their low yields under high-input conditions (Castiñeiras, 1992).

Following the disintegration of the USSR in 1989, the Cuban agricultural sector had to cope with a drastic reduction in input and trade support, shifting gradually towards more self-sufficient and rational forms of production.

Many remarkable technical and social transformations occurred as a response to this challenge. In the 1980s, Cuba had carried out 87 percent of its external trade under preferential price agreements, imported 95 percent of its fertilizer and herbicide requirements, and owned one tractor for every 125 ha of farm land. After the collapse of the socialist block, foreign purchase capacity was reduced from US$ 8.1 billion in 1989 to US$ 1.7 billion in 1993. This greatly affected the country's ability to buy agricultural inputs (Funes, 1997).

To address the crisis, the Cuban government implemented changes in all sectors to reduce the negative impact on the national economy. During the early 1990s, severe social and economic changes were made in order to maintain the social

guarantees of the government while simultaneously reconstructing the Cuban economy (Enriquez, 2000; Rosset and Benjamin, 1993). Cuba thus undertook one of the most dramatic changes in farming systems, having to move from being the highest agrochemical consumer in Latin America, to very-low-input agriculture in less than three years (Funes, 2002).

However, the plant breeding sector has been slower to adapt. Even though the professional plant breeders faced a difficult economic situation and researchers had few incentives, they pursued top-down approaches and adopted rigid reductionist perspectives. Within this existing system, the solution was not as simple as technology substitution. Due to the financial crisis, research institutions faced various constraints, such as lack of access to, or maintenance of, important genetic resource collections; energy blackouts; incapability to refresh seeds; and a decrease in the number of international programmes that had formerly supported Cuban research institutions in the 1990s. The national seed supply system urgently needed to expand, but lacked the financial resources to do so. In the 1990s, its seed production capacity for maize and bean had fallen by 50 percent (Ríos and Wright, 1999).

Through the informal system, the production of seeds of the basic staples of the Cuban diet became a major issue in many parts of the country. These genetic resources had provided a basis for plant breeders to select commercial genotypes during the industrial agriculture period. However, relatively little attention has been paid to this informal seed management system and much genetic variability had already been eroded (Esquivel and Hammer, 1992). Usually, the maintenance of genetic diversity was considered very close to environmental protection, with an altruistic rather than profit-making approach. The public plant breeding sector in Cuba and other Latin American regions considered agro-biodiversity management and plant improvement as an exclusive activity of professional researchers.

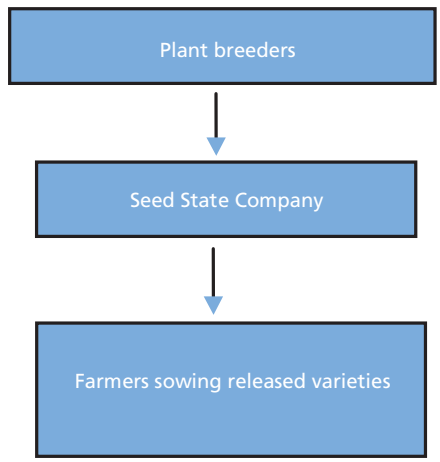
Making use of the space opened up by the economic crisis, a participatory seed dissemination programme emerged, inspired by some former work with pumpkins (Ríos, Soleri and Cleveland, 2002), and aiming to develop participatory seed production, improvement and distribution practices. This programme uses a variety of tools, including seed fairs and participatory variety selection, as strategies for seed diversification to improve the yield and genetic diversity in Cuba.

22.2 CHANGES IN THE PARADIGM: TOWARDS PARTICIPATORY SEED DIFFUSION

In principle, the Participatory Seed Diffusion (PSD) concept emerged in Cuba to integrate diversity seed fairs with farmer experimentation. A seed diversity fair is an approach where plant breeders, farmers and extension agents have access to diversity in one or more crops. Varieties from formal and informal seed systems are sown under the usual cultural practices of the target environment. Stakeholders have the possibility to make selections in the field. They do not know the seed sources of the varieties in the plot. After the farmers have taken and experimented with selected seeds on their own farms, discussions on varietal performance take place within the communities between farmers and researchers. This discussion is considered the start of the farmer experimentation period.

The two models – the centralized, conventional breeding model developed

FIGURE 22.1
The centralized seed diffusion model in Cuba



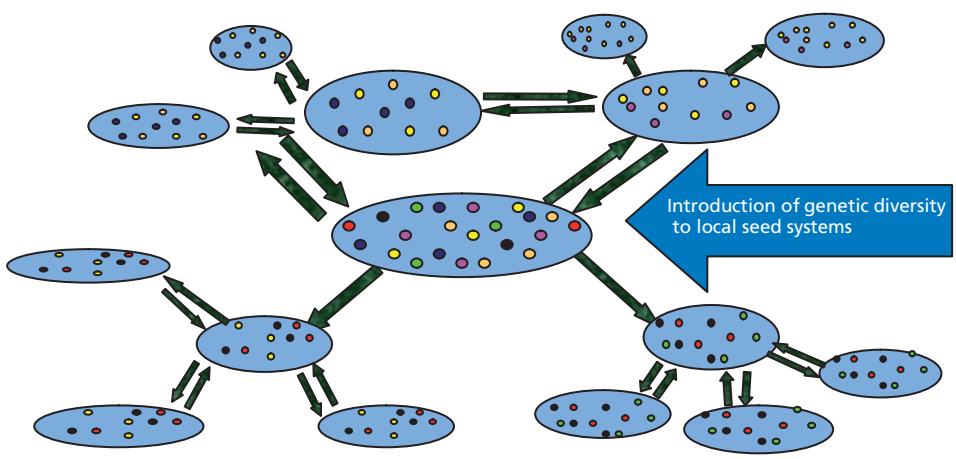
in Cuba during the 1980s, versus the decentralized, participatory plant breeding model – are shown in Figures 22.1 and 22.2, respectively.

In contrast to the centralized model, PSD is based on the individual farmer, through Agricultural Production Cooperatives, farmer experimenters, and groups or clubs, among other entities, which test and spread throughout the community varieties of high interest. Starting with the introduction of genetic diversity, through a process called chain reaction (Ríos, 2003), a diversity nucleus is built up that provides genetic diversity to others, and that grows exponentially through farmer participation. Once farmers see the favourable effects of experimenting with genetic diversity, they organize themselves into farmer research groups. Each diversity nucleus promotes knowledge, social organization and entrepreneurial centres characterized by intensive genetic flows and continued discussion around local innovation.

22.3 THE DIVERSITY SEED FAIR

The first diversity seed fair was held at the National Institute for Agricultural Science (INCA) in 1999, as an approach for disseminating maize seeds suitable for low-input agriculture (Ríos and Wright, 1999).

FIGURE 22.2
Participatory Seed Diffusion promoting a "Diversity Chain Reaction"



Source: Ríos, 2003a.

There, professional breeders provided farmers with access to diversity from the formal and informal seed systems, and the seeds were sown under relatively low input conditions (Ríos and Wright, 1999).

Some months before the first diversity seed fair, two breeders undertook maize seed collection missions to a farming community in the province of Pinar del Rio, and to Santa Catalina in Havana province. A selection was made for hardiness under low-input conditions, and 66 landraces (entries) were collected, including 10 from the focus communities in Havana province. In addition, four commercial varieties were supplied from research institutes. These were planted in December on an experimental plot at INCA. Each of the 70 lines was sown in three rows, and wide border strips were sown with a mixture of different lines.

Because of lack of financial resources, the experimental plot received only one irrigation treatment and no fertilizer or pest control inputs. Eighteen farmers from regions of high-input production, along with formal-sector maize breeders, social scientists from the National Agricultural Research System (NARS), and representatives from the National Small-Farmer Association and the former Cuban Association of Organic Agriculture (ACAO) attended the first seed diversity fair.

The farmers were taken to inspect the maize experimental plot and to examine cobs of all the maize lines from this plot, with each farmer selecting five preferred lines. Seeds from these lines would later be given to the farmers for experimentation. Short questionnaires were used to gather information on the farmer's evaluation of each line chosen, and the results were discussed. The main problems associated with seed management and use were low seed quality, low seed availability, and the incidence of pests and diseases. Availability of training and extension, exchange of seeds, and input availability were considered less problematic.

TABLE 22.1
Selection criteria for maize varieties, accepted as important by farmer participants

Criterion	Percent of farmer acceptance
Plant yield	87.5
Plant height	87.5
Positioning of leaves	62.5
Number of leaves	60.0
Leaf colour	45.5
Leaf size	41.3
Stalk width	76.3
Number of cobs	57.5
Ear colour	32.5
Ear size	40.0
Susceptibility to lodging	31.3
Cob weight	50.0
Cob height	40.0
Cob fullness	40.0
Husk colour	28.7
Cob diameter	37.5
Cob husk cover	55.0
Cob size	42.5
Cob shape	55.0
Insect damage	35.0
Cob length	45.0

Source: Ríos and Wright, 1999.

In the field, farmers rapidly selected from the large number of lines on offer. They showed an immediate preference for the mixed varietal border stands as these showed a better response to low input conditions than the mono-varietal rows. The importance of each of their selection criteria is shown in Table 22.1.

In the selection, 80 percent of the farmers identified different preference criteria for each of the five lines they had selected. Participants observed better results from

mixed-variety rather than single-variety planting, which led researchers to conclude that they would have to overcome contradictions in the practice of maintaining varieties through strict isolation, as demanded by the formal system.

It became clear that farmers looked not only at yield, but also valued aspects such as plant height, stalk size, number of cobs, and number and position of leaves. This is an indication of the need for alternative breeding objectives.

Selection criteria chosen for maize varieties indicated that farmers, in general, did not practice seed saving. In fact, during the discussion period, several of them asked how to save seeds.

The general reception given to this new participatory approach was positive, given that farmers were historically accustomed to a more top-down management procedure. Farmers had rapidly and easily selected between the 70 lines on show, and a very large range of new seed lines had been extended to them. The plant breeders who started to work in PSD felt that this diversity indicated the need to refocus seed management so that yields and cob quality could be improved under low input conditions (Ceccarelli and Grando, 2002; Ríos, 2003; Acosta *et al*., 2003; Martínez and Ríos, 2003b). Stimulating the flow of genetic resource variability has shown the potential available for increasing yield performance on trial plots for farmer acceptance.

22.4 FARMER ACCESS TO GENETIC DIVERSITY

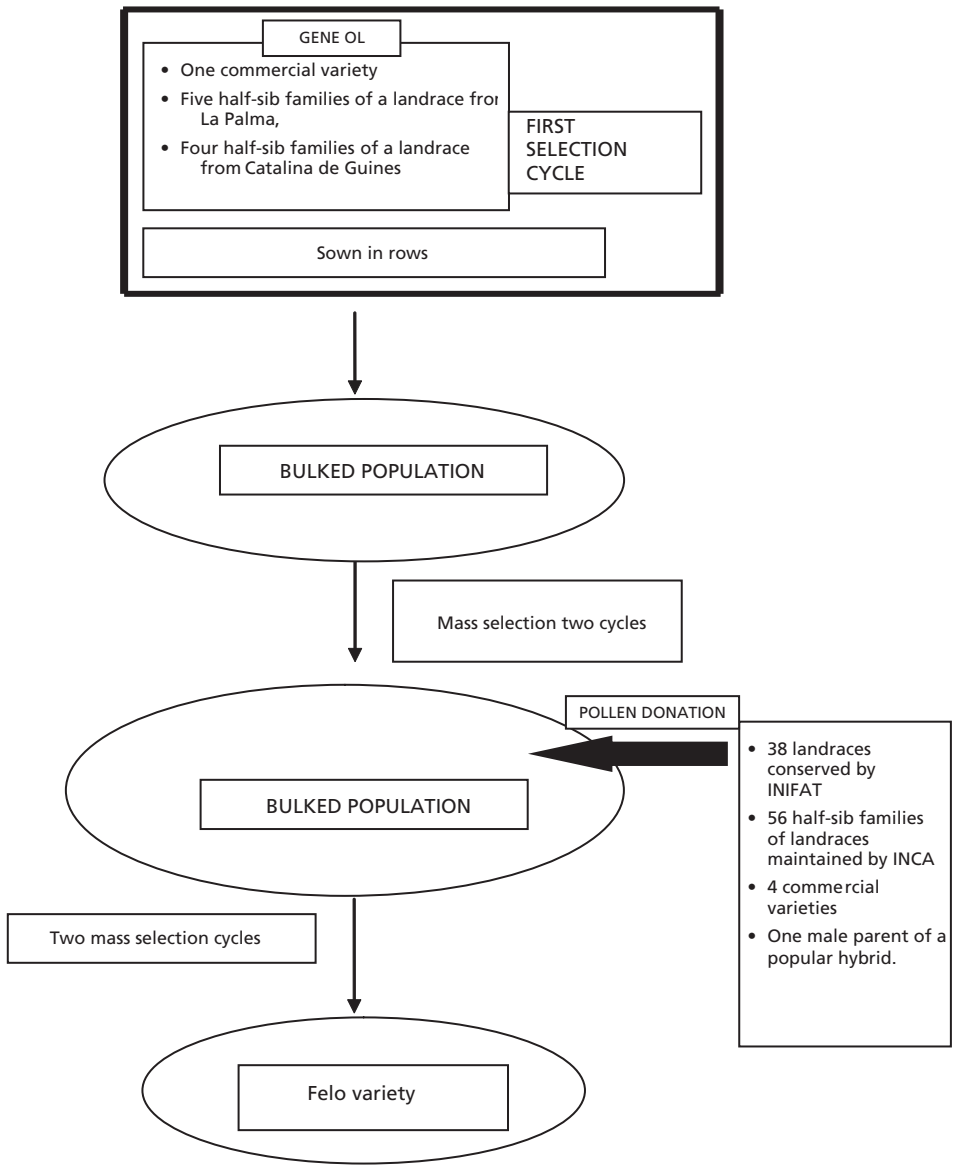
22.4.1 Cross-pollinated crops: the example of maize

Three months after the Diversity Seed Fair, the farmers' capacity to develop maize populations was assessed among nine farmers working on three cooperatives and one private farmer; all ten had attended the maize seed fair. Three of these farmers were unable to maintain their seeds because they lacked the conditions required for conservation from season to season, having relied for more than fifteen years on the formal seed sector, which supplied improved seeds every season.

The gene pool of the maize population of one Havana farmer who selected from the seed fair was found to be composed of different seed origins: one commercial variety from the formal seed sector, five half-sib families of a landrace from La Palma (a neighbouring province), and four half-sib families of a landrace from Catalina de Guines (a neighbouring municipality of the same province) (Figure 22.3). Later the same farmer bulked all materials and selected in the field the best 1 500–2 000 plants according to cob size, plant cob height and husk covering, during three cycles. Afterwards, at a seed fair prepared by his cooperative, this bulked population was sown along with 38 landraces conserved by the Fundamental Research Institute (INIFAT) gene bank, 56 half sib families of landraces maintained by INCA, four commercial varieties and a male parent of a popular hybrid (Ortiz *et al*., 2006, 2007).

Subsequently, the bulked population was named Felo (the nickname of the local farmer breeder) and two mass selection cycles were done. Gradually, this new seed pool, under farmer management, increased maize production and diffusion amongst cooperatives, and the area intercropped with maize increased over the years (Table 22.2). Maize rose from being one of the most neglected crops in the cooperative to the third important profitable crop (Ortiz *et al*., 2003a). Currently, this population, cv. Felo, is under seed multiplication and continued selection, having gained recognition from all

FIGURE 22.3
Maize selection scheme used by Felo – a farmer in Cuba



Source: Ríos, 2003a.

the municipality stakeholders, and has been registered as an official variety in Cuba.

Usually, the conventional model of breeding cross-pollinated crops entails recombining in the first stage of the breeding programme, and once breeders identify a certain population with desired characteristics, this population is maintained in isolation (Ríos, 2003). The interesting fact learned through the Felo experience was

TABLE 22.2
Maize production in Cooperative Gilberto Leon, Havana, Cuba

Year	1999	2000	2001	2002	2003	2004
Maize area (ha)	36	52	65	72	96	120
Maize area of seeds improved by farmers (ha)	0	10	65	72	96	120
Intercropping (ha)			25	50	60	

Source: Ortiz *et al.*, 2003a.

TABLE 22.3
Origins of bean varieties grown at seed diversity fair

	Commercial varieties	Genetic diversity conserved in gene bank	Accessions collected in the participant communities (Landraces)	Total
Black beans	17	30	16	63
Red beans	16	15	8	39
White beans	4	14	4	22
Total	37	59	28	124

Source: Lamin, 2005.

the possibility of improving yields by disseminating seed diversity. Each genetic pool built up by farmers could probably be continuously recombined, choosing for yield improvement as well as other important traits holding cultural or market values.

According to the first results of PSD in Cuba, seed diversity fairs should become a recombination process whereby farmers can have access to genetic diversity at community level. In this sense, farmer experimentation can play two roles, first in continuously providing the best progeny to the diversity gene pool at community level. and second in providing farmers with the opportunity to select the best recombined family in a certain cycle in the field. Thus, PSD in a cross-pollinated crop such as maize seemed to be a simple method where the recurrent selection principle can be applied (Maldonado *et al.*, 2006).

22.4.2 Self-pollinated crops: the example of beans

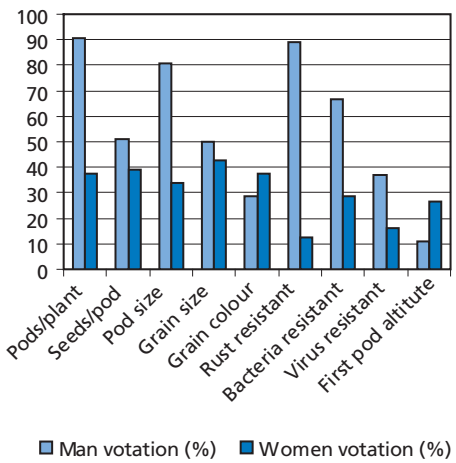
In the case of common bean, a self-pollinated crop, PSD in Cuba has been working mainly with released varieties and landraces, using a non-segregating population. Farmers could access up to 124 varieties of bean from different sources (Table 22.3) grown under low-input conditions at the INCA Experimental Station. Each variety was sown in a small plot, where participants could select up to five varieties to be taken home and tested on their farms under their prevailing production circumstances.

After more than half of the varieties had reached the stage of physiological ripening, a meeting was held with the farmers.

In the case of bean, farmer participants came from different biophysical and socio-economic contexts. Both marginal and industrial farming systems were represented by 42 farmers, as well as some NARS scientists, members of NGOs, functionaries and technicians of the Ministry of Agriculture.

The bean seed diversity fair was attended by male and female farmers. It was planned to carry out varietal selection for women and men separately (Verde *et al.*, 2003). A questionnaire was used in

FIGURE 22.4
Differences in plant selection according to gender focus



order to see whether there were differences in selection criteria according to gender. At the same time, 60 varieties were cooked and participants were grouped in small teams of 3 men and 3 women to evaluate 10 varieties each, with an extra questionnaire on cooking qualities to be completed by participants. Team members facilitated the processes of understanding and filling in questionnaires by participants.

Male farmers voted for varieties with high yield and associated characters, such as number of pods per plant, pod size and disease resistance. In contrast, female participants voted for varieties with large pods, grain size, shape and grain colour. Female farmers' criteria seemed to be more closely related to culinary properties than those of the males (Figure 22.4)

Most farmer participants associated grain colour with variety, and because of this it was interesting for farmers to see agro-morphological differences within colour in the first bean diversity fair; they commented on the degree of variability of disease resistance within the same colour (Miranda, 2005).

At the beginning, the selection exercise was run on an individual basis; however, some farmers collectively decided to chose a wide range, as they wanted to test a range of varieties in their region. They were keen on organizing a seed diversity fair exercise in their own communities. During the selection exercise in the field, the team noted that none of the farmer participants had previously had the opportunity to gain access to genetic diversity.

In the cooking test, males noted that more than 80 percent of the varieties tested had good quality, whereas females showed more rigour in testing beans for cooking quality (Table 22.4).

After the bean seed selection, the project focused on supporting experimenter farmer networks as had been initiated for maize. In the case of bean, the mission was to compare and release varieties according to the farmers' traditional farming systems. Workshops on experimental designs were held at community level. Experimenter farmers' networks started to grow at community level, the reaction of farmers

TABLE 22.4
Gender comparison of cooking quality in common bean

	Male (n = 100)			Female (n = 80)		
	Good	Medium	Bad	Good	Medium	Bad
Flavour	80	13	7	63.7	26.3	10
Softness	95	3	2	73.8	21.3	5

Source: Verde *et al.*, 2003.

confronted with bean diversity was overwhelming, and nobody expected genetic diversity to be of such importance to farmers.

In fact, the main interest of farmers in maize and bean was to be able to select amongst the wide range of varieties according their own criteria. Numerous varieties conserved in the gene bank showed good performance even though some had been lost off the official varieties list. The spirit of experimentation, the opportunity for more such productive options, and the gender differences detected in the first participatory seed selection exercises in Cuba, inspired farmers, scientists and others stakeholders to further explore PSD in Cuba and abroad. Consequently, a Mexican and Cuban team started to collect seeds from different sources, promote diversity seed fairs and farmer experimentation in their local context.

22.5 COLLECTION OF SEED DIVERSITY

A collecting mission was carried out as a multidisciplinary effort. Teams composed of scientists from INCA and local stakeholders, in Cuba and Mexico, collected beans, maize and rice landraces in different provinces and municipalities (Table 22.5).

In terms of the results of these diversity collection missions (Ríos *et al*., 2006), the teams in Cuba, La Cuenca del Papaloapan and Chiapas reported potential interesting material for certain breeding programmes. In general terms, the farmers donated their seeds freely. In the case of Mexico, the phenotypic diversity of collected seeds of maize was enough to organize different plots in both Chiapas and La Cuenca de Papaloapan. In Cuba, an important bean collection was donated by the Fundamental Research Institute in Tropical Agriculture (INIFAT), and rice germplasm was donated by the Rice Research Institute (IIR), in addition to collected material.

For maize, most of the diversity collected in Mexico came from local seed systems, with 8 lines provided by CIMMYT. In Cuba, most collected maize came from local seed systems, with only four commercial varieties coming from professional breeders. In every case, each maize, bean and rice accession collected per family farm was considered as a variety. In comparison with maize and bean, only very narrow rice diversity was found in the field (Moreno *et al.*, 2003).

In Cuba, several public organizations were very open to providing materials for seed diversity fairs, and these have been considered an important support to the PSD process. The main problem in Cuba was the resistance of conventional plant breeders to facilitate segregating populations.

In Mexico, it was extremely difficult to break the barriers for access to public germplasm for developing seed diversity fairs at community level. At the same time, the reaction of some public plant breeders was conservative.

22.6 FARMER'S ACCESS TO GENETIC VARIABILITY

The genetic diversity conserved in conventional gene banks, accessions collected during the collecting mission undertaken by the project, and commercial varieties donated by breeders of bean, maize and rice, were sown in 2001 in Cuba at farm level. In La Cuenca del Papaloapan (a catchment covering the tropical area of Oaxaca and Veracruz states), Mexico, two seed diversity fairs were held for maize and bean, and rice plots were attempted but it was not possible to obtain a harvest (Table 22.6).

TABLE 22.5
Characteristics of collection missions

Crop	Region	Number of accessions	Number of farmer donors	Number of municipalities involved	Number of communities involved
Maize (*Zea mays* L.)	Cuenca del Papaloapan	204		11	43
	Chiapas Highland	368	221	20	66
	Cuba	254	82	25	65
Beans (*Phaseolus vulgaris* L. & *P. coccineus* L.)	Cuenca del Papaloapan	52	48	8	20
	Chiapas Highland	201	125	19	40
	Cuba	150(1)	—	—	—
Rice (*Oryza sativa*)	Cuenca del Papaloapan	8	2	3	4
	Chiapas Highland	3	2	2	2
	Cuba	16	15	2	8

NOTES: (1) 60 accessions were donated by INIFAT gene bank.

TABLE 22.6
Location and number of varieties grown in seed diversity fairs in the 2002–2003 period in Mexico and Cuba

Diversity plot location	Crops and no. of varieties per location	Farmers selecting varieties	Altitude (masl)	Experimental field plot topography
Chenalho, Chiapas, México	Maize: 84 Beans: 75	37 in maize; beans could be harvested owing to high rainfall regime.	1500	Heterogeneous
Comitán, Chiapas, México	Maize: 139 Beans: 74	No growth because of drought.	1600	Homogenous
San Cristobal de Las Casas	Maize: 95 Beans: 68	49	2120	Homogenous
Ejido Valle Nacional, Municipality Santa Maria de Jacatepec	Maize: 131	163	40	Homogenous
Doroteo Arango Municipality Acatlan de Perez Figueroa	Maize: 97	100	54	Homogenous
San José de las Lajas. La Habana, Cuba	Beans: 70	42	132	Homogenous
San Antonio de los Baños, La Habana, Cuba	Beans: 97	35	150	Homogenous
La Palma, Pinar del Río, Cuba	Beans: 53	81	60–80	Heterogeneous
Los Palacios	Rice: 80	41	60	Homogenous

Source: Ríos *et al*., 2006.

In Chiapas, four experimental plots were cultivated with collected genetic diversity: at Villa Flores Agriculture University in the lowland, and the other three in the highlands of Chiapas at La Albarrada (San Cristobal de Las Casas Municipality), Yabteclum (Chenalo Municipality), and Comitan (Comitan Municipality). In the case of Mexico, most of the maize diversity grown in the different places was mainly donated by farmers. Consideration was made of the altitude where the seed was collected, in order to avoid mis-adaptation.

All cultivation of the diversity plots was undertaken according to the traditional practices of the participant communities, except in Chiapas lowlands and Cuenca del Papaloapan, where a half-technical package was applied. Each accession collected was considered a variety. In all diversity plots, farmers were allowed to choose five or six varieties to take home.

22.7 PARTICIPATORY PLANT BREEDING AND SEED PRODUCTION

In both Mexico and Cuba, the facilitation of farmers' genetic diversity through seed diversity fairs increased the early reaction obtained from the first two seed diversity fairs carried out in Cuban communities. In Chiapas highlands, only one seed diversity fair was held, the other three did not reach harvest due to drought or flood.

In every place where seed diversity fairs were held, farmers showed great interest in introducing greater genetic diversity into their own farm system (Table 22.7).

In Mexico, participants appreciated that some traditional varieties were grown in seed diversity fairs. In this way, traditional varieties which had almost become extinct were chosen and multiplied by participants.

After farmers took seeds to be grown on their farms, different workshops were conducted to discuss selection methods at community level and experimental design principles. In La Cuenca del Papaloapan, the follow-up process in maize was focused in two communities: Doroteo Arango and Vega del Sol.

In Doroteo Arango, after one selection cycle working with professional breeders, the farmers had to move off their land because of conflicts of land tenure, and so their maize breeding programme was completely halted as all the farmers' efforts had to be oriented toward land recovery.

In the other community, Vega del Sol, germination of distributed seeds was poor with farmers losing all the varieties selected at the fair, so then the farmers and professional breeders decided to start a new collection mission in their communities. They collected 91 accessions in neighbourhood communities, setting up four experimental plots, one per colour.

After three years of mass selection, farmer participants had sown 17 ha of land with four maize gene pools: white, yellow, red and black, choosing the best cob each cycle. Farmers from the community started to make some negotiations with tortilla

TABLE 22.7
Genetic diversity chosen by farmer participants in the seed diversity fairs

Place	Crop	No. of participants	No. of varieties grown in seed diversity fair (b)	Chosen diversity (a)	Percent effective diversity (a/b × 100)
San Cristobal de las Casas	Maize	51	84	51	60
La Palma, Pinar del Rio	Beans	74	52	47	90.4
Ejido Valle Nacional, Municipality Santa Maria de Jacatepec.	Maize	163	131	91	69.5
Doroteo Arango Municipality Acatlan de Perez Figueroa	Maize	100	97	70	72.2
San José de las Lajas	Beans	42	70	46	65.7
San Antonio de los Baños	Maize	35	97	47	48.5
Los Palacios, Pinar del Río	Rice	41	80	60	75

Source: Ríos, 2005.

companies to provide maize for specialized markets.

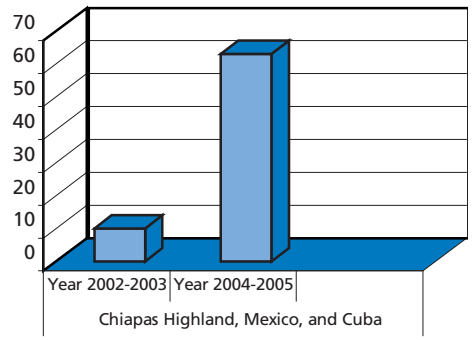
The General Farmers and Workers Union (UGOCP), which was coordinating PSD in La Cuenca del Papaloapan, had since the 1980s lead an Agrarian Reform, and its members were facing strong conflicts over land tenure. Once the farmers had land, UGOCP needed different approaches for enhancing rural development more independent from external resources. Indeed, involving farmers in plant breeding meant a new, more civil approach and orientation for UGOCP for the enhancement of local innovation and participation in making agriculture more sustainable.

PSD was an attractive initiative not only for farmers but also for technicians, researchers, functionaries, politicians and policy-makers, who learnt about the opportunities offered by genetic diversity for cropping systems using less agrochemicals, and about their and its relationship with indigenous knowledge. In practice, PSD showed to be a concrete approach for improving farming systems with interesting entrepreneurial opportunities.

In Chiapas and Cuba, the process developed so fast that the number of seed diversity fairs increased exponentially in rural and urban areas (Figure 22.5). Simultaneously, the number of different crops grown increased from 1 in 2001 to 18 in 2004.

In the particular case of Cuba, PSD in the period 2003 to 2008 increased from three communities in the western part of the country to a national group of practitioners. This means that training programmes could be designed and implemented with the participation of local stakeholder to strengthen local seed systems. Master in Sciences projects and PhD programmes have been implemented in the communities, with local universities

FIGURE 22.5
Number of farmer's experimental plots in Cuba and Chiapas



Source: Ríos, 2005

starting to integrate their research work with farmer experimenter networks.

In rice cultivated under high and low potential environments in Cuba, farmers grew different varieties selected in seed diversity fairs. Interesting evidence has been reported by Moreno *et al.* (2005) and Lopez *et al.* (2005), who proved that varieties unpopular in seed diversity fairs had been officially promoted by the conventional seed system. In fact, PSD was adopted by the Popular Rice Movement as a national strategy to enhance rice genetic diversity to fulfil the different biophysical and socio-economic demands of popular rice growers in Cuba (Aleman, 2005, *Arroz con amor se paga*, video).

In Chiapas, Mexico, the process was initially introduced by UGOCP, and afterwards, the Development Secretary of Chiapas Highlands and the Indigenous People's Secretary of Chiapas endorsed the PSD approach as a key alternative for enhancing indigenous culture in the current social life of Chiapas State. During the scale-out process, two main reactions emerged: one where farmers were willing to start experimenting with varieties as

never before to rescue maize and bean landraces in Chiapas; the other where economic support was requested to grow experimental plots. The second reaction appeared to be conditioned by other rural programmes, which supported subsidies for food production in the region. Some farmer leaders in favour of the second reaction decided to pull out of PSD.

In Cuba and Mexico, according to the perceptions of the participants, yields have improved in crops under the farmer experimentation process, and farmers were able to diversify and disseminate varieties to the rest of the communities after three years of testing (Lamin, 2005).

In general terms, the amount of seed produced by farmers increased exponentially in the participating communities.

22.8 DECENTRALIZED SEED PRODUCTION SYSTEM

After four working years, the research team noted some differences in seed production concepts between PSD and conventional plant breeding. In PSD, a defining characteristic is the integration within the household or community of genetic resource conservation, plant breeding, seed production, crop production and food consumption. In contrast, in conventional plant breeding, these functions are institutionalized, specialized and separated (Ríos, 2003; Cleveland *et al*., 2005). Therefore, most of the farmers working with PSD test genetic diversity and subsequently multiply their best options to fill different demands from the family, neighbourhood and local market.

In marginal and industrial environments, the tendency was to retain as much diversity as possible. The reaction of some farmers from marginal environments in keeping diversity was: "We need to keep various options because who knows how hard is the next season" (A. Alda, pers. comm.; Mohamed, pers. comm.). Through PSD, farmers reinforce seed production to be exchanged for experimenting next crop season or simply for culinary testing, and they use seeds for promotion or in barter for other products. In some cases, farmers who never grew seeds are selling seeds to farmers or to the state seed company. Unfortunately, the team has no details of the volume of seeds sold through PSD.

Actually the official scheme of releasing certified seeds to be adopted by farmers has partially broken down. In PSD, as in other participatory plant breeding methods, farmers adopted varieties by experimentation, and released their best options once disseminated varieties were certified (Ceccarelli, 2005, pers. comm.). In this sense, the seed production process in centralized plant breeding, with no participatory element, officially starts when improved varieties are multiplied and certified for dissemination. In PSD, because farmers are participating in the process of selection from the beginning and they are continuously accessing genetic diversity, seed production is an integral element of the process through which farmers decide the varieties or crops that have to be multiplied and disseminated.

Currently, four agrobiodiversity centres have been built by collaborative efforts between farmers and professionals scientists in Cuba, to promote diversity through diversity seed fairs, farmer experimentation and seed production by farmer decision. Primary diversity centres are farms with capacity to introduce, test and disseminate genetic diversity.

The speed at which PSD has spread in Cuba and Mexico has caused an interesting conflict: on the one hand, the legislation does not allow free national seed flow

because seeds are not certified, and on the other hand, national food security depends on informal seed production in both countries. Therefore discussions to reconcile the differences are taking place in both Cuba and Mexico.

22.9 FARMERS' GENETIC GAINS

As yields were increasing in the communities, a discussion emerged in different communities implementing PSD about the real influences of farmer selection on yield response. In fact, the team and scientific community looked for hard evidence on farmer selection efficiency.

In conventional breeding programmes, one of the common indicators for determining the impact of selection consists of estimating genetic advance through selection (Falconer, 1960), which is described as follows:

$$S = h^2 \times DS$$

where: S = selection advance; h^2 = heritability and DS = selection differential, as discussed in detail in Chapter 2.

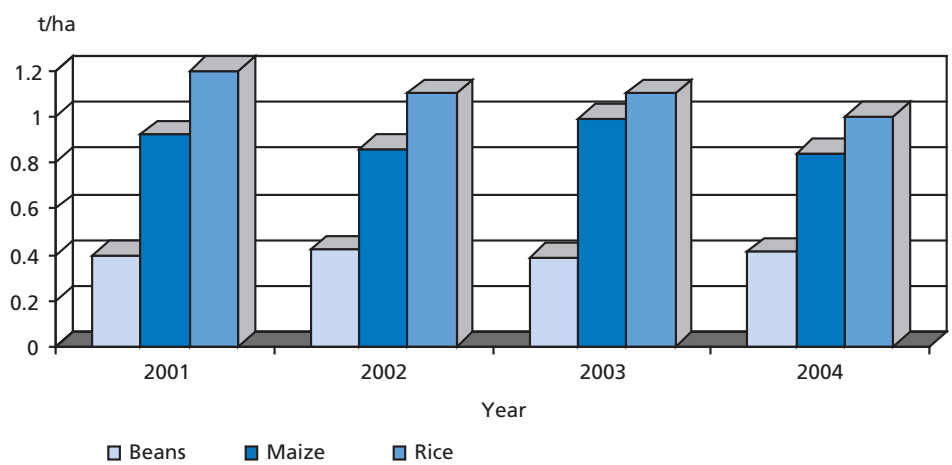
In the case of PSD, such estimation has been applied to each grower who has selected varieties during diversity fairs (Figure 22.6).

Indeed, the differential selection reached by farmers gave evidence of their capacity for obtaining superior materials amongst certain populations. The results strongly imply that farmers participating in plant selection and seed diffusion could collaborate in simultaneously increasing yields and diversity. In practice, access to diversity in the form of released varieties and segregating populations could provide an interesting fit at local level (see Rosas, Gallardo and Jimenez (2006) for segregating populations).

Other interesting evidence is the case of pumpkin breeding (Table 22.8). The farmers who choose gene pools on farm, according to their criteria, had more efficient use of energy for producing food and more profitable crops

Conventional pumpkin breeding in Cuba provides an example of the possible negative economic effects when

FIGURE 22.6
Average selection differential attained by farmers in 18 bean, 10 maize and 6 rice seed diversity fairs in Cuba



Source: Ortiz *et al.*, 2005.

TABLE 22.8
Economic impact of pumpkin breeding under low input conditions

Indicators (calculated as averages)	Varieties bred under high input conditions sown in low input conditions	Varieties bred and sown under low input conditions
Cost per ha under low input conditions (Cuban pesos)	702.3	708.3
Fruit yield (t/ha)	1.5	6.7
Total income (@ 0.16 Cuban pesos per kg)	240	1080
Net income per ha (Cuban pesos)	-462(1)	372
Benefit:cost ratio	0.34:1	1.5:1

NOTES: (1) average net loss.
Source: Ríos *et al.*, 2002.

TABLE 22.9
Socio-economic and biophysical contexts of scaled-out Participatory Seed Diffusion

	Indigenous culture	Farmer literacy	Research-development policy priority	Production potential
Republic of Cuba	Low	High	Public sector	High-Low
Cuenca del Papaloapan	High	Low	Private sector	High
Highland Chiapas State	High	Low	Private sector	Low

varieties are selected in an environment not representative of the target area. The occurrence of a cross-over response (Ceccarelli *et al.*, 1994; Ceccarelli and Grando, 2002) suggests the importance of having a realistic view about who will be using the products of plant breeding.

The experience described in this chapter attempts to maximize the role of local multi-sectoral efforts, including international, national and local stakeholders, through promoting the generation of benefits at local level by using PSD.

22.10 SCALING UP PARTICIPATORY APPROACHES

As a result of the outcome of the two breeding cycles in Cuba, the team and other partners decided to expand the pilot experience from the western part of Cuba in the form of a PSD programme for the central and western parts of Cuba, and to the Highland of Chiapas and La Cuenca del Papaloapan, Mexico. The working team was eager to know how PSDs, emerging from the western part of Cuba, could be practically adapted to other Cuban zones and abroad, with different biophysical and socio-economic contexts (Table 22.9).

What did we scale out? Chiefly we scaled out:

- The diagnostic phase, looking for local genetic diversity, intervention entry points and enabling institutional environments, for a change of paradigm.
- Seed diversity fairs in maize, bean and rice, to stimulate varietal demand and enhance farmer participation in generating benefits.

It was very effective to discuss the idea of PSD with a wide range of stakeholder participants; in fact, a constructive reaction was received from government, civil society and farmers. They built up the different teams and planned the activities, and immediately started to work. Local organizations were extremely cooperative in supporting the process.

The teams' main work objectives were to understand the seed flows, leadership relations and reaction of local policy-makers in terms of supporting the idea. In parallel, and as a key activity, teams collected genetic diversity from the formal and informal seed sector, mainly of maize and beans.

In addition, Cuba had a Popular Rice Movement which was highly suited to the application of PSD. The Popular Rice Movement is a people's movement to produce rice under low input conditions for self-consumption and markets within Cuba. This movement aiming at producing the main staple food emerged in the 1990s in response to the collapse of conventional rice production handled by the large state farms. Farmers were then allowed to plant rice everywhere, and the government made the land available for this (Moreno *et al*., 2005).

In terms of farming approaches, in Cuba, farmers were experiencing a 'special period' due to the collapse of the Socialist Block in the late 1980s (Ríos, 2003), which in general terms meant that they had very limited access to agrochemicals and improved seeds of basic grains. In Chiapas, in contrast, upland farmers had no choice but to grow their crops in a marginal environment. In comparison, La Cuenca del Papaloapan was a high-potential environment and had received enormous agricultural investment in the 1980s for maximizing yields according to Green Revolution philosophy. In 2001, however, farmers in this region had, for various reasons, lost a major part of the official financial support.

According to the diagnosis phase carried out before the PSD intervention, farmers who have more diversity and dynamic seed exchanges in maize had more profits, in both Cuba and Mexico. The experimentation capacity of farmers seemed to be an important element for successful family business under restricted financial conditions (Ríos, Soleri and Cleveland, 2002).

In maize, a cross-pollinated crop, there were significant agromorphological differences between farmer-collected accessions, even though the local maize population had the same name: criollo, pintico, amarillo, negrito, blanco, etc. One hypothesis is that such diversity made it possible to improve certain complex characteristics, such as yield, through farmer participation (Acosta *et al*., 2003; Martinez, 2005). In the case of beans, a self-pollinated crop, few bean types existed in industrial farming systems, and in certain lowlands of Chiapas farmers decided to stop growing beans due to disease attacks, whereas in the upland it was possible to collect different types of beans to be intercropped with maize.

In general terms, with beans, farmers perceived increased disease susceptibility and loss of genetic diversity over the previous decade. Limited access to new genetic diversity from either the formal or informal seed sectors was evident. Some morphological differences were found to be limiting genetic diversity within grain colour of farmers' beans prior to the PSD intervention (Miranda, 2005).

Finally, the team's work showed that the situation for Cuba and Mexico was common in terms of limited access to financial resources to buy seeds and agrochemicals for the production of basic grains. In the particular case of Mexico, stakeholders felt threatened by the USA policy of selling cereals at very low prices. In fact, the limited economic situation faced by Cuba, in relation to Green Revolution concepts, was not exclusive; other regions were suffering from similar problems and local innovation was emerging as a response for overcoming obstacles to producing food.

22.11 EXTERNAL COSTS OF PARTICIPATORY APPROACHES

Apparently PSD seemed to be an attractive process for local stakeholders; however, after four years of PSD implementation, one important question emerged: What will happen once PSD is no longer financially supported by external donors?

One of the key discussion points about public innovation systems in agriculture is in regard to financial support. NARS have been losing funds, and the international core budget of the CGIAR centres has fallen over the last 14 years (CGIAR, 1990–2004). As a consequence, both national and international institutes have been forced to be more innovative in their activities in poor regions.

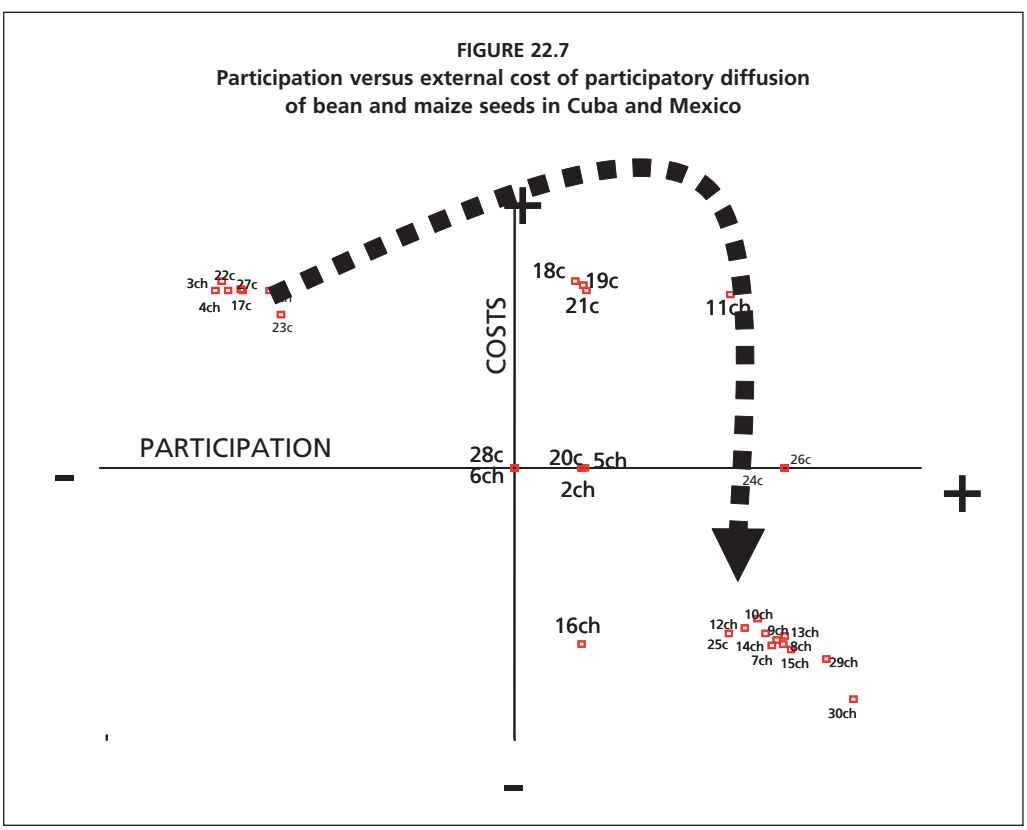
Taking this into account, the team estimated the external cost tendency and its relationship with the participatory approach in PSD.

An analysis of participation and external costs was carried out for all the maize and bean seed diversity fairs organized in Cuba and Chiapas over the last four years. To reach a better understanding of the relationship between participation and external costs, a graph (Figure 22.7) represents the two components plotted.

In the x component, participation was represented by different categories as follows:

- *Very high*: Farmers organized seed diversity fairs on their farms with varieties and technologies brought by themselves, they were able to involve communities in undertaking participatory approaches.
- *High*: Farmers organized seed diversity fairs on their farms with technologies

FIGURE 22.7
Participation versus external cost of participatory diffusion of bean and maize seeds in Cuba and Mexico



and varieties brought by professional researchers, farmers, NGOs, private companies, etc. Farmers were able to involve communities in undertaking participatory approaches.
- *Medium*: Farmers organized seed diversity fairs on public property, and seeds and technologies were supplied by farmers and professional researchers; farmers were partially able to call on participants for undertaking participatory approaches.
- *Low*: Public or private institutions organized seed diversity fairs on experimental stations, and researchers, extension agents, public or private functionaries took decisions. Farmers could not involve other farmers in undertaking participatory approaches.

The y axis was represented by three categories of external costs as follows:
- *High*: The expenses for food, participant transportation and implementation of diversity plots was covered by the project.
- *Medium*: The food expenses and participant transportation was paid for by the project. The expenses of implementing diversity plot was covered by communities.
- *Low*: The implementation of experimental plots, food and transportation was covered by the communities.

Figure 22.7 shows how the external cost decreases with an increase in participation over the four years of project implementation. The results show that external costs could be reduced gradually when local stakeholders adopt participatory methodologies, and the recognition of farmer knowledge as well as the economic benefits of farmer experimentation seems to be an important incentive for developing PSD. Farmers decided to incorporate trials as organic components of their farming systems.

The PSD in Chiapas was largely focused on the highlands, with farming systems on sloping areas, and with farmers having very low literacy levels. However, most of the characteristics represented by the high participation and low external support in Figure 22.7 belonged to the seed diversity fairs developed in that region.

The results confirmed the hypothesis that local innovations are not strictly related to literacy levels. Even though farmers had a high literacy level in Cuba, the relationship between professional scientists and farmers was weak before the collapse of the socialist countries, and it was currently taking some time to establish a new relationship. It has been a difficult process to convince the professional scientists to consider farmer participation as a scientific element of their profession.

In general terms, the agricultural education systems did not consider farmers as collaborators or partners of research work, scientific services or policy-making, and decisions in agriculture had a very strong top-down character. However, research institutes and development organizations have worked directly in different ways to quickly adopt participatory plant breeding methodology, even though the concept was not well documented. Personal influences of researchers have played a critical role in scaling-out PSD (Chaveco *et al*., 2006).

22.12 CONCLUDING REMARKS

Usually, the route of plant genetic resources collected in communities ends at research institution gene banks, to be used in conventional plant breeding programmes (Almekinders *et al*., 2000). The experiences discussed in this chapter provide evidence of how material from collecting missions could be tested, multiplied, improved and disseminated by farmers and local

stakeholders. In practice, PSD maintains landraces by using farmer experimentation. Traditional varieties were re-evaluated within local and national contexts.

Due to the progress of seed diversity fairs and farmer experimentation, farmers in Cuba and Mexico started to add diversity to their farming systems with additional species. They were able to organize seed diversity fairs, simple experimental designs on-farm, and diffuse diversity among themselves, in their communities and to professional scientists. Farmers were able to produce seeds to be distributed.

Interesting combinations of cropping systems with new and old crops and new technologies emerged from the collaborative efforts. Currently, two instances have emerged so far: hundreds of concentrate formulas for animal feeds were built up from the collaborative efforts promoting agrobiodiversity enhancement and farmer participation (Ponce and Rodriguez, 2005, pers. comm.).

Recently in Chiapas, technical education is being organized with farmers using more than 30 seed diversity fairs, and the University of Villa Flores is implementing some maize breeding protocols in different regions of Chiapas State (Espinosa, 2005; Aguilar, 2005, pers. comm.).

Professional scientists actually doubted the capacity of farmers to simultaneously manage four or five trials of different crops, but finally they realized that farmers had a more profound conception of their farming system than had been imagined by professional scientists.

Conventional plant breeding has an enormous capacity for diversity generation in major crops. Moreover, powerful selection methods for fixing important genes into certain populations are undertaken by international and national research centres. However, the explicit aim of reaching wide geographical areas is a limiting factor when developing capacity for seed diffusion in diverse biophysical and socio-economic contexts. In this sense, organizing farmers into local innovation groups can maximize local, national and international efforts.

To consider only conventional research and development organizations as partners in plant breeding could be underestimating other strong forces for driving demand and having positive impact in rural and urban areas. Public and private innovation initiatives need to involve farmers and other local stakeholders as a key forces for agricultural benefit.

In fact, the PSD has been a continuous learning process in action. The professional breeder participants become more efficient in their interventions, and farmers more precise in their experimental systems, so it is crucial to enhance collaboration between farmers and scientist-technicians for generating and sharing benefits at community level. The action of the project has been able to influence the inclusion of the PSD concept into the education curriculum, nurturing new, critical students capable of combining biological and social sciences in Cuba and Mexico.

The institutional participants noted that involving farmers in the process of plant selection helped to recognize the enormous value of diversity generated by national and international centres as well as the genetic diversity managed by farmers. Before PSD, national scientists had few collaborators and limited impact from their work. However, currently and because of the increasing demand for genetic diversity, they have hundreds of collaborators multiplying local, national and international efforts in diffusing genetic diversity.

Currently, the public research institutions are suffering from severe financial restrictions; they are strongly influenced by external budget changes, which are very vulnerable to socio-economic or political changes. The field experience described in this chapter provides a clue that genetic diversity could lead to a viable, small, economic initiative for many local stakeholders.

New institutional arrangements for enhancing collaborative efforts between scientists and farmers seem to be an important issue in reaching a better understanding of local seed systems and agrobiodiversity incentives (Vernooy, 2003) as 'development cells' for national and international development.

It is quite clear that the experience accumulated from PSD in Cuba and Mexico shows that innovation in agriculture is not exclusively a business for professional scientists, but that by involving local stakeholders and farmers the impact of plant breeding in different contexts might increase. PSD has been able to revive the professional plant breeding role and farmer knowledge in a current context. Perhaps the results obtained by the collaboration of farmers and scientists, and the difficult economic situation faced by national and international public plant breeding, could facilitate new approaches towards more diverse, productive, socially and economically fair plant breeding in future years.

The economic and energy efficiency of selecting varieties under real environmental conditions, and farmers' attitudes to experimentation, become important arguments to convince policy-makers to apply PSD as a transformative tool in agriculture. Officially, PSD has been focused as a method to encourage public welfare and re-evaluate public institutions in Cuba. At the same time, the organizations leading PSD in Mexico are focusing on more entrepreneurial tendencies to show how people marginalized by top-down approaches can be recognized as innovators and potential local managers of plant genetic resources. In practice, both country cases are dealing with their own contexts. However, both countries are enhancing diversity, farmer participation and new technological and institutional arrangements towards more integrated food production.

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CHAPTER 23

Towards new roles, responsibilities and rules: the case of participatory plant breeding

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23.1 INTRODUCTION

This chapter discusses three interrelated topics: the roles of the people involved in new, participatory plant breeding (PPB) approaches; the type of research management process that best guides these approaches; and a number of institutional issues that influence the space for doing things differently. These three topics will be illustrated with concrete examples of new practice from around the world. New plant breeding approaches were developed in order to do things differently, complementing and providing an alternative to conventional plant breeding. Hence, the focus of this chapter is on practice. However, we argue that this new practice could benefit from theory, and that many interesting and valuable theoretical insights are available. Brief mention will therefore be made of a number of relevant theoretical insights from fields such as participatory learning and action research, development studies, and organizational development studies. At the same time, we also hope that the new practices presented here inform and advance participatory plant breeding theory.

23.2 PARADIGM SHIFT

As we have argued elsewhere (Vernooy, 2003; Vernooy and Song, 2004), a new scientific practice is warranted to address persistent rural development issues such as food security, biodiversity conservation, environmental management and empowerment. This also affects crop science. Conventional plant breeding in most countries has been and remains largely centralized. Key research decisions are made at the top of the organizational hierarchy: Which crops to focus on? Which researchers to fund? and Which methods to use? Experiments take place at one or a few experimental stations. Variety release requires approval from a central body, and seed regulations are defined centrally. This practice is characterized by top-down decision-making and information flows. Farmers or others interested in variety diversity and improvement have little or no meaningful say in the process. The research process is very much inward oriented and often disconnected from farmers' experiences of the diverse and often rapidly changing environment(s) on which they depend.

This kind of practice is informed by reductionist thinking. This implies two main things. First, reductionist measurement fails to take into account the multiple and interrelated variables that farmers rely on to judge the value of a crop and cropping system. These farmer variables are often, if not always, site- and season-specific, embedded in particular genotype-by-environment (G×E) variations, informed by social variables such as gender, class and ethnicity, and influenced by socio-economic factors, such as market access and access to services such as credit, research and extension.

Second, conventional crop research tends to disregard local biodiversity, or at best considers it very instrumentally: as inputs for breeding, and best maintained *ex situ* in the proximity of the breeding station. It neglects the importance of biodiversity at the landscape and agro-ecological levels. If you reduce agrobiodiversity you weaken the resilience of agro-ecosystems and their capacity to deal with change. When this happens, communities face more limited options in managing their land and resources. The end result is that opportunities for the creation and re-creation of farmer knowledge and experimentation – the very processes that are essential for agro-biodiversity conservation, evolution and

improvement – are lost. This relationship between social and biological diversity is often overlooked (Vernooy, 2003).

Conventional crop research is also positivist in nature, seeking the accumulation of objective knowledge through the production of empirically testable hypotheses. This paradigm is mirrored in a so-called reproductive learning perspective (van der Veen, 2000) that assumes that there is a body of objectively verifiable knowledge and that this can be taught by breaking down content into its essential elements. Such a perspective has serious limitations. An alternative is provided by a social constructionist perspective that views the role of science as the creation of concepts or theories that expand flexibility and choice (Röling, 2000). This view postulates that all social action is open to multiple interpretations, none of which is superior in any objective sense.

Social constructionist learning assumes that important features of the external world are uncertain and disputed, and that people actively construct their understanding of it. Rediscovery and innovation, not repetition, are essential parts of this construction process. In practice, researchers and development workers often assume roles as facilitators, rather than instructors. They encourage work in groups and shared planning, action and reflection. A social constructionist perspective also can be informed by transformative learning (van der Veen, 2000). In this approach, learners together build a more integrated or inclusive perspective of the world. Through the learning process they jointly transform some part of their worldview, for example, their understanding of social relations in their own community. Manifestations of transformative learning in natural resource management include, for example, new values or patterns of decision-making that farmers generate and apply outside the immediate arena of the learning intervention.

23.3 INTERACTIVE ROLES

From a practical point of view, the foregoing implies working toward a new division of labour, new partnerships and new forms of decision-making and learning. PPB approaches developed during the last decade have made significant inroads into giving concrete shape to these new roles and responsibilities. One of the goals of PPB is to involve farmers in the research in ways that are meaningful and useful to them, improving the quality of their participation as a means of empowerment. Farmers are no longer just the passive (end-of-the-line) recipients of technologies, seeds and information. In participatory approaches, they are encouraged to take on active roles, help set direction, and take part in decision-making. Women farmers in particular have a priority place because they often have intimate knowledge of crop production and reproduction. They often also have particular needs and interests in food security, and play leading roles in households, extended families and social networks.

Participatory approaches focus on meaningful, fair and iterative interaction. From a decade of PPB experience around the world, we know that all this is easier said than done. PPB requires a lot of effort. Concretely, it means that those who take the initiative to practise PPB, be they originally (more) farmer or (more) scientist-driven, need to pay special attention to:

- Getting to know the various people involved, and building trust.
- Getting to understand and respect different (and sometimes initially opposing) perspectives, interests and expertise.

- Acknowledging personal, social and institutional constraints to collaboration.
- Communicating clearly and in a timely manner.
- Finding common ground through discussion, reflection and negotiation.
- Defining tasks to be accomplished and agreeing on who will do what and when up-front, e.g. setting objectives; selecting germplasm to be used; choosing breeding, propagation and selection methods; selecting sites where the research will be carried out; identifying the type of end-product to be produced; and agreeing on the means by which the product(s) will be distributed (i.e. benefit sharing).
- The time and effort that any change process requires, and the often very slow pace of change in everyday life.

These points imply exploring the practical meaning of participation, its potential and limitations.

23.4 PARTICIPATION: INTENT, DECISION-MAKING, CONTEXT

There are many ways in which participation in a research cycle can be organized and managed. Participation is a normative concept and implies argumentation and negotiation, and sometimes contestations and struggles over knowledge, intent, interests, direction, results and benefits. Whether we practise participation in a project setting or as part of a broader development process, it means having to deal with politics: Who defines the agenda? Who makes decisions? Who reaps the benefits? Who is included or excluded? Participatory research can take a variety of different forms in terms of who participates, how and when, and who decides about what, how and when. The forms it takes also depend on context. In the case of a research project, this context includes the organizational set up, but also the wider societal configuration, including the economy, policies and laws, and the social make-up. After all, research endeavours do not operate in a void. A useful typology of participation is the following:

- **Contractual participation**. One social actor has sole decision-making power over most of the decisions taken in a research process. Others participate in activities defined by this social actor in the sense of being formally or informally 'contracted' to provide services and support.
- **Consultative participation.** Most of the key decisions are made by one social actor, but emphasis is put on consultation and gathering information from others, especially for identifying constraints and opportunities, priority setting and evaluation.
- **Collaborative participation.** Different social actors collaborate and work on a more equal footing, emphasizing linkages through an exchange of knowledge, different contributions and a sharing of decision-making power during the innovation process.
- **Collegial participation.** Different social actors work together as colleagues or partners. 'Ownership' and responsibility are equally distributed among the partners, and decisions are made by agreement or consensus among all, from identification of the research problem or opportunity, through to final assessment.

It is useful to differentiate between types of participation in order to understand how this influences research results. 'Community' participation in research can be differentiated according to the level of control over the process (who sets the agenda), when (at what stage of the research), and according to the nature of representation (who speaks for whom). We conclude this section by arguing that

there is no right or wrong amount, or a single manifestation of participation. It depends on intent. Participation is always a social product, i.e. it emerges from people interacting and joining forces in practice. The actual process and outcomes depend on many factors and will be shaped and sometimes constrained by unforeseen events. Outcomes sometimes include unintended consequences, some perhaps considered negative, some perhaps positive. To illustrate some of the points made so far, we present the first case study.

23.5 CASE STUDY 1: NEPAL

In the late 1990s, the non-governmental organization (NGO) Local Initiative for Biodiversity Research and Development, better known as LI-BIRD, based in Pokhara, Nepal, undertook a study in the low hill region of Nepal to document and analyse farmers' knowledge of upland rice (*Ghaiya*) varieties. A team of one plant breeder and four agricultural technicians carried out the study, with the involvement of men and women farmers of five villages where local Ghaiya diversity was predominant (Joshi, Rana and Subedi, 2001). The study was done and directed by the LI-BIRD team using techniques such as resource and social maps (through transect walks), participants observation, interviews, group discussion, and the collection of farmers' preferred varieties.

At the same time, the team initiated a so-called participatory landrace selection process, similar to a participatory variety selection (PVS) process. In this case, selection concerned landraces from the region collected and selected by the research team instead of modern varieties that are often used for PVS. The landraces were selected by the team on the basis of the results of the documentation study, i.e. to match farmers' interests in particular varieties or traits in varieties, such as drought tolerance, grain quality and yield potential on poor soils. These were the breeding variables about which farmers were most concerned. The research team designed the outline of the subsequent experiment, in which a number of farmers took part in testing the newly introduced varieties.

The LI-BIRD team decided how to distribute seeds, how many, and to how many farmers. Farmers themselves decided where to test the varieties received, how to grow them, and with which varieties to compare them. The team later documented and analysed these farmer decisions. During various stages of the cropping cycle, the research team documented farmer assessments of the new varieties, individually and collectively, using questionnaires, farm-walks and group discussions. The collective assessment served as a means to interact with all the farmers about their experiments.

The research team concluded that this process of participatory landrace selection was an effective means of broadening the range of suitable Ghaiya landraces available to farmers, at little risk to them and at a relatively low cost to the researchers. Farmers were able to evaluate new options under their own farm conditions, observe results at other farms, and to come to useful conclusions in a relatively short time (two years of experimentation). LI-BIRD also concluded that now that this methodology has proven effective, it should be easier to use it in the future, given that costs per unit would be lower. In particular, given that there is very little institutional support for Ghaiya rice, this would have great merits for (poor) farming communities. LI-BIRD and partners in Nepal continue to build on this experience, expanding it to other sites as well as to other crops.

Working *in situ*, and decentralization

The Nepal case study points to a number of important features. Perhaps the first to note is that the LI-BIRD research team worked *in situ*—on farms and in communities—with farmers as research colleagues, each complementing as much as possible the other's knowledge, skills and experience. In this case, the research project was and remained strongly LI-BIRD directed, as the team decided where to work and also maintained a generally strong hand in directing the research process, i.e. selection of varieties to be tested, seed quantity, and number of farmers invited to grow the 'new' varieties. These decisions clearly affected the results. Although farmers benefited from introduced varieties, it is likely that their relatively limited decision-making restricted the potential for a more transformative change. (This is an observation about the relationship between intent and result, and should not be seen as a critique.)

Another feature that emerges from the case study is that decentralization replaces centralization as the main organizing principle in order to address specific local contexts, i.e. G×E interactions, and socio-economic variables including age, class or caste, gender and ethnicity. Although the research described took place at only one site, as a means to validate the approach, LI-BIRD subsequently concluded that this principle of decentralization could be used on a wider scale, and probably countrywide. Again, here we are dealing with a researcher-directed intervention, but one that could have potentially a much broader impact as it concerns an organizational principle at the programme, and even national research policy, level.

Decentralization (see also Chapter 9) has been at the heart of many alternative approaches, but, as with participation, it comes in many forms and degrees. The International Centre for Agricultural Research in the Dry Areas (ICARDA) participatory plant breeding efforts in the Middle East and North Africa are based on it. One of the advantages it offers in terms of efficiency is that selection in farmers' fields avoids the risk of useful lines being discarded because of their relatively poor performance at experimental stations, where conditions are almost certainly more favourable. Decentralization as an organizational practice could be looked at with the same perspective as participation.

23.6 CASE STUDY 2: ICARDA

This study is adapted from Vernooy (2003), and based on various ICARDA research results and publications.

In the late 1990s, a team of researchers at the ICARDA pioneered a new way to work with farmers in marginal rainfall environments of Morocco, the Syrian Arab Republic and Tunisia. They set out to work together with farmers and aimed to fulfil the needs of people living and working in the harsh conditions of the region. In Syria, for example, researchers worked with 'host farmers'. In the context of Syrian farming, these were men who accepted the invitation made by the researchers to partake in the research in nine communities (identified by the researchers) and with two regional research stations. These host farmers and their neighbours, varying from a few to a dozen or more, took care of the trials, which involved experimental lines from the research station and the farmers' own varieties. Farmers and breeders assessed the results independently in successive trials from 1997 to 1999. Several promising new varieties were identified from these trials.

It quickly became apparent that the farmers' selection criteria, largely based on

environmental factors, were quite different from those used by the national breeding programmes. To the surprise of many, the selections made by the farmers were at least as effective as those made by the breeders. The newly introduced materials gave good yields, and this in areas where plant breeding had not previously been successful. Farmers also gained access to varieties that responded to preferred traits such as tall plants, large kernels, good early growth vigour, high tillering and lodging resistance. Seeing these promising results, breeders quickly adopted the new ideas and attitudes, becoming supporters of the participatory approach and expanding it to other areas and to other crops. The team learned that earlier plant breeding programmes were ineffective on marginal lands because they seldom included among their selection criteria those traits that are important to farmers.

In addition, it became clear that decentralized selection in farmers' fields avoids the risk of useful lines being discarded because of their relatively poor performance at experimental stations, where conditions are almost certainly more favourable, through fertilization or irrigation, for example. Decentralized selection combined with farmer participation from the initial stages of the breeding process is a powerful methodology to fit crops to specific biophysical and socio-economic contexts, and to respond to farmers' needs and knowledge.

The researchers learned a number of other critical lessons from the project. Among them is the fact that farmers can handle a large number of lines or populations, or both. Most notably, in Syria in phase 2 of the work, the number of lines assessed in some villages increased from around 200 up to 400! In fact, farmers warmly welcomed the ability to select among a large number of lines; some farmers have started seed increase of selected varieties. This has opened the window to a more dynamic process, with new materials being introduced at any time.

The researchers also noted that women's selection criteria often differed from the men's, highlighting the importance of ascertaining when and why they differ. They also noted that farmers became empowered by their involvement in the research process, gaining the confidence to take decisions on crosses as well as on factors such as plot size and the number of locations. Perhaps of equal importance to the researchers themselves, the project revealed the need for specific training in areas such as experimental design and data analysis suitable for situations where the environment (a farmer's field under farmer management) cannot be under the scientists' control as it is in the research stations. ICARDA and national partners have continued to expand their efforts by scaling-up the approach in the national systems in the region and by trying out the methodology on other crops.

Research management

What becomes apparent from the above discussion and case studies is that such new approaches require a different way of organizing time, labour and the research process, i.e. the roles and responsibilities previously described. The emphasis is on step-wise producing or co-producing as effectively and efficiently as feasible 'a project' through face-to-face interactions, especially in the field. Bringing different disciplines to the table and field is one important element. Research management requires flexibility. It is not about implementing blueprints. This new method

of organizing time and labour will therefore benefit from adaptive process management knowledge and skills. Farmers usually already have a significant amount of this capacity, and it is useful to build on their expertise, and perhaps, where useful, explore ways to strengthen it. Researchers may need to be trained to acquire this capacity. Insights from learning theory can be of much value, as well as from participatory monitoring and evaluation approaches.

Start-up periods of collaborative research are usually very labour intensive, requiring a good deal of time and effort to lay a foundation of trust and to build working relationships, both within the research team, and between the core team and others involved in the research. Longer-term commitments are important, to be able to create meaningful and effective collaboration and to cope with unavoidable setbacks, such as a crop failure due to drought. Experiments, particularly in plant breeding, usually require various cycles of selection to produce useful results, and thus time horizons should not be too restricted. Organizing regular feedback opportunities and using the results promptly to adapt or change directions is another important element.

23.7 CASE STUDY 3: CUBA

The study is adapted from Vernooy (2003), and draws on National Institute for Agricultural Sciences (INCA), Cuba, research results and publications.

In 2000 an interdisciplinary group of dynamic researchers at INCA took on the challenge of reshaping agriculture on the island. They began a project designed to improve the yield and quality of the maize and bean crops in both unfavourable and more favourable production areas, through a combined effort of increased varietal diversity and strengthened local farmer organizations. The project is already making an important contribution to improving Cuba's food security options.

The key element in the project has been to involve the farmers, and this has been achieved through farmer research in experimental groups. The project team believed that strengthening the organization of farmers increases their capacity to experiment and innovate and to make stronger demands on the formal agricultural research system. One method the researchers used to introduce farmers to new or unknown varieties or lines was the seed fair. Initially, this took considerable planning and facilitation efforts as fairs were organized by the INCA team and at the INCA station. Farmers were wary of this new approach (none had ever visited the INCA station), but many attended out of curiosity. What they saw overcame their reservations. The researchers managed to collect genetic materials for many maize and bean varieties (later, fairs were organized for other crops), including commercial and local varieties, as well as promising new lines. The farmers were impressed.

The fairs demonstrated to farmers the diversity of their staple crops. The researchers subsequently allowed the farmers (men and women) to select materials for testing in their own fields, under local conditions. This proved very popular and successful. It proved that farmers are able to assess and select from a large number of options alongside breeders. Ultimately, the fairs have proved to be hugely popular, so much so that farmers quite spontaneously started to organize similar fairs in their own communities. Initially, the researchers guided and supported the farmers in doing this, but subsequently farmers organized fairs mostly or all by themselves. Farmers, breeders and extension agents now continue

to rub shoulders at fairs, assessing varieties, and selecting the ones they like best. Breeders continue to assist farmers with experimental design on-farm, but all trials are adapted to the local context.

Farmers say that in addition to introducing new and higher yielding maize and bean seeds (e.g. bean yields in the Havana experimental site have gone up on average by 15 percent and in the La Palma site by an average of about 35 percent), some of which are also more resistant to diseases, the fairs provide new knowledge about how to handle and conserve seeds. By developing closer links between farmers and researchers from the formal system, the fairs have also increased the farmers' capacity for experimentation. And last, but by no means least, the fairs have become social and cultural events that bring rural people together, young and old, and give them an opportunity to share their knowledge and experiences.

The project team also organizes regular field days as another way to learn more about farmers' preferences. Here the farmers, both men and women, are interviewed about their preferences. The information gathered is crucial for the INCA plant breeders in identifying parental materials and selection criteria. To date, the project has been successful at both broadening the genetic base and improving the quality of varieties. INCA is currently extending the methodology and results to other provinces through collaboration with other Cuban agricultural research entities. Envisioned is the creation of a national network to exchange experiences, new ideas and seeds, and to provide inputs into the policy-making process.

Interdisciplinarity and facilitation

The case studies presented so far indicate that interdisciplinarity is desirable. Understanding natural resource and crop dynamics requires taking into account both the biophysical and the social dimensions of the processes involved in managing and maintaining productivity and agrobiodiversity. Plant breeders have much to gain from working with social scientists in an interdisciplinary research mode to document and analyse the social nature of farming, plant breeding, and of doing research. Social scientists have the opportunity to ground their work in real-life situations.

Facilitation and convening are new and important additional roles for traditional plant breeders. Additional training is an important investment if these skills are lacking (among researchers or farmers, or both). Working with a diverse group of people – including scientists in various fields, women and men farmers, and extension workers – means balancing a variety of ideas, interests, skills and personalities. Managing the process of participatory planning, implementation, monitoring and evaluation means paying significant attention to interactions and communication, as well as ensuring openness and fairness. Building and strengthening the participatory process becomes a central part of the agenda. The following case is a good example.

23.8 CASE STUDY 4: CHINA

This study is adapted from Vernooy (2003), Vernooy and Song (2004) and various Center for Chinese Agricultural Policy (CCAP) documents.

In China, new plant breeding approaches have been pioneered by CCAP, a leading agricultural policy research institution that is part of the Chinese Academy of Sciences (CAS), and by the Guangxi Maize Research Institute (GMRI), part of the Chinese Academy of Agricultural Sciences (CAAS). The CCAP/GMRI research aims to

identify technical and institutional options for developing more effective linkages and mutually beneficial partnerships between the formal and farmers' seed systems. The main hypothesis is that only such new institutional development can enhance sustainable crop development. and *in situ* and on-farm management of genetic resources. It also aims to strengthen women and men farmers' research and management capacities to maintain agrobiodiversity in the specific Chinese context.

A major PPB project was implemented in Guangxi province in south-west China following an impact study carried out from 1994 to1998 by the International Maize and Wheat Improvement Centre (CIMMYT) to assess the impact of CIMMYT's maize germplasm on poor farmers in south-west China. That study critically analysed the processes of technology development and diffusion. One of the key findings of the impact study was the systematic division between the formal and the farmer seed systems. This resulted in inadequate variety development, poor adoption of formally bred modern varieties, an increasingly narrow genetic base for breeding, and a decrease in genetic biodiversity in farmers' fields.

The project team supported farmers' groups through training, linkages and network building, and market involvement among farmers and with the formal system actors. Policy-changes aim to bring about conceptual change among formal research and seed system actors so that they better understand farmer roles and enable farmer participation. The project is implemented by a team of women and men from various institutions and groups, from different disciplinary backgrounds and operating at different levels. Five women farmer groups, six villages, six township extension stations, two formal breeding institutes and CCAP have been directly involved in project design and implementation. The team is engaged in an ongoing dialogue in order to integrate the very many contributions from the very broad expertise base. This is not always easy, but so far efforts have been very productive.

The research uses a participatory plant breeding methodology adapted to the local context. Trials in six villages and on-station have included both participatory plant breeding and participatory variety selection experiments. The trials allow for comparison in terms of locality, approach, objectives and the types of varieties tested. Varieties include landraces, open-pollinated varieties, so-called waxy maize varieties, and varieties introduced by CIMMYT. Some of the CIMMYT varieties have been locally improved through crossings and selections. Agronomic traits, yields, taste and palatability of these improved varieties are satisfactory. They are showing better adaptation to the local environments. Varietal diversity is increasing.

The project's PPB field experiments, both in farmers' fields and on station, have been functioning successfully as a platform to involve the main stakeholders from both formal and farmer systems. They have facilitated effective interaction, communication and collaboration among them. Through this platform, the approach and results have reached high-level decision-makers (at the provincial and national levels), and some inroads have been made into the policy process. Farmers, women in particular, are now speaking up in meetings and expressing their ideas, needs and interests. In a still strongly top-down research and policy environment, this represents a major change. PPB has also strengthened the local-level organizational and decision-making

capacity of farmers. Groups of (mostly women) farmers have started to define specific support that they would like to receive from the extension service.

They have put forward the idea of initiating seed production and marketing, in particular of pollen variety maize seeds. Marketing research is underway in Guangxi and neighbouring provinces. The aim is to add value to the women farmers' produce. This is expected to make the on-going activities and process of PPB and agro-biodiversity management more sustainable. In addition, following the organization of a first successful diversity fair in 2003 in the township, they are now planning follow-up fairs in their villages, and possibly in the city of Nanning, the provincial capital. They plan to sell theirs seeds at these fairs.

Creating an enabling environment: institutional issues

Roles and management process questions lead to the consideration of a number of institutional issues. Perhaps the most important ones are incentives and rewards that recognize and value promising and successful efforts. Perhaps the basis for all PPB approaches involves two tenets: farmers have a key role to play in crop improvement; and farmer-researcher collaboration can produce added value that farmers or researchers alone could never realize. Acknowledging and institutionalizing these two tenets then becomes paramount. But there are other institutional issues of importance. Farmers should be officially recognized as 'co-authors' of new varieties and recognized in publications that document PPB processes and final results. Plant breeders should be recognized and rewarded not only for the release of new varieties, but also for their contribution to the process leading to the final products. Increasingly, so-called access and benefit sharing issues are moving to centre stage. This theme is discussed in more detail in Chapters 9 and 24.

Research policies and grants should be targeted to proposals that deal adequately with process management questions, including the redefinition of roles, as outlined above. This means nothing less than a shake-up of most organizational practices, rules and regulations. Creating an enabling environment will therefore take time and effort. Although projects, with clear time and resource boundaries, have an important role to play to try out new ways of doing things, changes will be required that go beyond projects and must become embedded in everyday practices. This kind of change will probably not come easily, and could be frustrated by vested interests and opposing powers. Setbacks are to be expected. Accepting and fostering a learning-by-doing approach is still very novel.

The key organizational capacities required for promoting and supporting new approaches include staffing; infrastructure, technology and finances; leadership; management; and linkages and networking (Horton *et al*., 2003). In many countries, organizations (be they part of the NARS, NGOs or Community-Based Organizations), have difficulties in sustaining, let alone strengthening, these capacities. Moreover, in several countries, there are numerous and often vast regions where there is no organizational presence at all for rural development. The challenge then becomes to look for alternatives (see the following and final case studies).

Other, very important, institutional issues relate to seed systems, at both the local and informal levels, as well as the national and formal levels, where one has seed regulatory frameworks dealing with

varietal and seed quality; variety release systems regulating the spread of varieties of proven quality to farmers; phytosanitary law; seed certification schemes that aim to control varietal identity and purity; and seed quality control mechanisms that check viability, purity and health. This theme is discussed in more detail in Chapter 21.

These so-called regulatory framework components are embedded in broader societal institutions, including policies affecting rural development and agricultural research more broadly, e.g. land tenure, taxation, marketing, financing of public research, provision of credit, and provision of extension services. Depending on context, research into these broader institutional questions may be highly relevant. The current trend of shrinking budgets around the world for public national agricultural research seems to make this area particularly relevant.

Looking for opportunities to build on local change processes already in motion or to explore spaces for change becomes an important skill. The following case studies are examples of how spaces for change were found or created.

23.9 CASE STUDY 5: HONDURAS AND NICARAGUA: CREATING SPACE FOR EXPERIMENTATION, ENHANCING LOCAL ORGANIZATIONAL CAPACITY

The Honduras case builds on Humphries *et al.* (2005), and the Nicaragua case on Vernooy *et al*. (2000) and Vernooy (2003).

Local agricultural research committees, or CIALs to use their Spanish acronym, have sprung up all over Latin America. CIALs bring farmers and researchers together in a process of joint experimentation and learning. The concept was developed at the International Centre for Tropical Agriculture (CIAT) in Colombia, and it quickly caught on. They vary in size and characteristics, but they all have one thing in common: they provide a direct link between locally organized farmers and the formal agricultural research systems.

Honduras

In Honduras the number of CIALs has grown rapidly and there are now 82, comprising around 900 farmers in different regions of the country, most of them in remote mountainous areas where they are frequently excluded from conventional agricultural research and extension services. The CIALs are organized into five regional associations of a national CIAL federation, the Honduran Association of CIALs.

Fifty-five of the farmer research teams are supported by a Honduran NGO, La Fundación para La Investigación Participativa con Agricultores de Honduras (FIPAH), which began as a project entitled Investigación Participativa en Centroamérica, which was supported initially by CIAT and then by the International Development Research Centre (IDRC) between 1995–2000; since 2000 it has been supported by a Canadian NGO, USC-Canada, under its Seeds of Survival (SoS) Program, with financial backing from the Canadian International Development Agency (CIDA).

With a team comprising four local agronomists with the collaboration of a Canadian rural sociologist, FIPAH's agronomists have successfully bridged the divide between plant breeders at the region's largest agricultural research institution, La Escuela Agrícola Panamericana, Zamorano, and poor hillside farmers (Humphries *et al*., 2005).

Achieving organizational integration between farmers and scientists in Honduras is quite remarkable. In the countryside there are few strong community organizations

available to national and regional institutions seeking to support local development, and local social capital has frequently been characterized as low. Thus FIPAH had to basically start organizing from scratch. Following years of regional conflict and military repression, local people were generally distrustful of group endeavours and building up the CIALs has required very strong facilitation skills. Farmers had to learn to trust the agronomists and their own capacity to undertake research, often in the face of local ridicule concerning the small size of the test plots. This necessarily took some time and therefore was not without cost. However, as CIAL members' research has grown to include the testing and evaluation of a broad range of technologies and, more recently, the successful improvement of local maize and bean landraces, they have earned their communities' admiration. Local CIALs are now supported by a group of farmer facilitators, local CIAL experts, who have increasingly taken over regular support to the CIALs from FIPAH agronomists. Today, the FIPAH agronomists mainly play a backstopping role behind the scenes, supporting the regional CIAL associations and farmer facilitators.

For plant breeders at Zamorano, the skill sets in agricultural innovation-testing and development that CIAL members have acquired present an extraordinary research opportunity. The plant breeders are now in a position to reach into remote agricultural areas, far from the experiment station, where they have never been able to work before. The recent results of participatory bean breeding, conducted both on-station and in farmers' fields, showed how different the choices made by breeders and farmers in marginal agricultural areas can be: none of the materials selected by the breeder at Zamorano was subsequently chosen by farmers once these were added to farmers' own F_6 trials.

Zamorano breeders who were once sceptical of involving farmers at an early stage of plant breeding, when segregation of materials is underway, are now convinced that farmer researchers are better placed than they are to decide what seeds work best in communities where biodiversity is high and where small socio-economic differences between families can lead to very different choices of technologies. This has led Zamorano breeders to conclude that the best strategy is to provide such farmers with a diversity of segregating materials as well as advanced lines to allow them to select what is best for them (Rosas, Gallardo and Jiménez, 2003). In addition, as Zamorano provides agricultural research support to countries throughout Central America and the Caribbean, recognition of the importance of participatory research as complementary to conventional breeding represents a considerable step forward in conceptual terms.

The final step is to engage the different CIAL Associations and their members in scaling up the PPB varieties. At the present time, Macuzalito, an improved, small red landrace bean, released by the CIAL Association of Yorito, Victoria and Sulaco in August 2004 (Humphries *et al.*, 2005) is being tested in the different CIAL regions prior to being multiplied up for wider use in the near future. A strong federation of farmers' organizations is vital if PPB is to have an impact beyond the locality where it was originally conducted.

Nicaragua

In one region of Nicaragua, a CIAT research team initiated a process of CIAL formation in 1999. The initial assessment

of the organizational context in this region (the Calico river catchment) revealed on the one hand that very little formal agricultural research was carried out in the area or that the results of research carried out elsewhere (by the NARS) reached the area; on the other hand, it was learned that farmers themselves were not known to experiment very widely.

The CIAT team hypothesized therefore that there would be space for the implementation of the CIAL methodology in terms of providing a tool for farmer experimentation and strengthening of the organizational processes in the area. CIALs could create new groups or could build upon existing groups, and introduce new roles in the community, such as by providing a service through doing research for and with the community, opening the door to participatory decision-making, problem diagnosis and experimental design, and establishing new communication patterns among farmers and between farmers and external agencies, such as through CIAL-led presentations, field-days, and direct demand for support directed to outside agencies from the NARS.

The CIAT team also thought that CIALs could be players in changing the very much supply-driven mode of operations of most NGOs in the area into a more demand-driven one, as well as getting government agencies and universities interested in the area and problems and needs of farmers. The core idea behind the CIAT team efforts to initiate a process of CIAL formation was to provide local communities with a (new) way to carry out research collectively, focusing on and solving a locally felt natural resource management problem (to be identified through participatory problem analysis), and thus further enhance local organizational capacity.

At first, two CIALs were formed; over the years the number grew rapidly. Several committees have since moved on to experimenting on a larger scale, addressing new aspects of problems in their communities, such as soil fertility. A number of new farmer-leaders have emerged, including several women. Where possible, CIALs are linking to each other to exchange ideas and results within the catchment and beyond, through participation in the annual CIAL meetings in Honduras, for example. They also are building bridges to formal research and technology organizations in the country.

The Honduras and Nicaragua experience suggest that positive change is possible despite very difficult institutional contexts. Through sustained efforts, new organizational forms can emerge, a demand-driven research process can be set in motion, and useful linkages can be developed with and between local, farmer-led initiatives and national or international units, expertise and resources. These changes do not come about easily, and set-backs have been numerous. However, the CIALs are contributing to revitalizing rural innovation and to defining many new rules for the research and development game.

23.10 SYNTHESIS

This chapter has addressed three interrelated elements of the division of labour in participatory plant breeding: the roles of the people involved, the nature of the research management process, and a number of institutional issues that influence the space for doing things differently. Underlying these three elements is the need to pay attention to:

- Getting to know the various people involved and building trust.

- Getting to understand and respect different perspectives, interests and expertise.
- Bridging these perspectives, interests and expertise through an interdisciplinary, iterative, learning-by-doing approach.
- Acknowledging the very real personal, social and institutional constraints to collaboration, and actively finding ways to overcome them.
- Communicating clearly and in a timely manner.
- Finding common ground through deliberate planning, monitoring and evaluation efforts.
- Defining tasks to be accomplished, jointly and up front, and agreeing on who will do what and when, e.g. setting objectives; selecting germplasm to be used; choosing breeding, propagation and selection methods; selecting sites where the research will be carried out; identifying the type of end-product to be produced; and the means by which the product(s) will be distributed.
- Recognizing the time and effort that any change process requires, including the often very slow pace of change in everyday life.
- In other words, recognizing the need to explore the practical meaning of participation, its potential and its limitations.

PPB experiences to date, including those documented in this chapter, suggest that significant progress has been made in terms of the development of an alternative and complementary approach. This has not been without difficulties, constraints and setbacks. New challenges, such as scaling up (e.g. institutionalization) and scaling out (e.g. application and adaptation to more favourable production environments), have emerged and are now being researched in a number of countries, involving farmers, researchers, extensionists and policy-makers. These efforts tell us that organizational and institutional questions, such as those addressed here, are central to (participatory) plant breeding, deserving as much attention as more technical issues.

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CHAPTER 24

Breeders' rights and IPR issues

Susanne Somersalo and John Dodds

24.1 INTRODUCTION

Since Gregor Mendel in the 19th century laid the foundations for genetic improvement of crops and animals, several technologies have been successfully applied to improve characteristics of the crops. The improved plant breeding methods include among others cell culture techniques, mutation breeding and hybridization. Genetic modification of plants is one of the main milestones in plant breeding techniques during the last decades of the 20th century. Along with transgenic plants came the need to identify and detect genes and their products. Genome mapping and proteomics are the new areas of research, which are of importance also to modern plant breeding.

Traditionally, plants and plant varieties have been treated as common property. However, in the beginning of 20th century plant variety protection (PVP) arose by means of intellectual property rights (IPR). Originally, the need for protecting new varieties was raised by the breeders of ornamental plants. The Plant Patent Act of United States of America was implemented in 1930 to protect vegetatively propagated plants, excluding tuber crops. In the Netherlands, the Breeders' Ordinance was enacted in 1941, and Germany enacted its Plant Variety Protection legislation in 1953. The first International Convention for the Protection of New Varieties of Plants (The UPOV Convention) was signed in Paris in 1961, and established the International Union for the Protection of New Varieties of Plants (UPOV).

The rationale for PVP is to provide an opportunity for breeders to gain returns from the investment made in developing a new variety. It is also believed that protection may stimulate private sector investment and facilitate technology transfer, thereby benefiting the framers and consumers. Another voice has been raised, arguing that protection ruins the tradition of farmers having the right to save and exchange seeds, thereby forcing farmer dependency on seed companies.

Along with the development of plant breeding methods, the means to protect the innovations have diversified. Not only is there a need to protect the improved crop varieties, but there is also a need to protect the methods of producing these varieties, the genes incorporated in them and the gene products that are known to give the plant its specific character. Furthermore, there is a need to protect databases containing information on the improved genes, and a need to protect methods for use of certain characteristics of improved crops, for example.

Generally speaking, the prime form of intellectual property (IP) to protect technical innovations is a patent, while the most well known means to protect plant varieties is plant breeders' rights (PBR). Recently, other forms of intellectual property, such as copyrights, trademarks and trade secrets, have also become important, not only in other fields of life sciences but also in plant breeding.

During the recent decades, the plant breeding industry sector has changed a lot: a traditionally public funded sector is today fairly much privatized. Furthermore, during the era of globalization, most countries have joined the World Trade Organization (WTO) and thereby are under duty to respect several international treaties regulating various aspects of trade and industry, including intellectual property. Despite the international frames set by various treaties, countries still have lot of flexibility in terms of enforcement. Furthermore, the breeders are still left with various means to control newly developed varieties, research results and so on.

In this chapter we shall first introduce the different means to protect intellectual property. We shall then discuss international treaties and conventions providing the frames for intellectual property legislation of the member countries. We shall also shortly discuss the alternative ways of protecting intellectual property by contracts, material transfer agreements (MTAs) and physical means to prevent unauthorized use of improved germplasm.

24.2 FORMS OF INTELLECTUAL PROPERTY

A breeder can choose today from a menu of different IP protection options. The following sections introduce the basic forms of protection.

24.2.1 Plant breeders' rights

The best known form of IP in plant breeding is plant breeders' rights (PBR). Often *sui generis* protection is mentioned in connection with PBR. *Sui generis* means 'of its own kind' or 'special', and *sui generis* protection refers to protection of plant breeders' rights with forms other than patents.

The best known known *sui generis* system is the one that is provided under the UPOV Convention. Under UPOV, PBRs are called Plant Variety Protection (PVP).

As of 9 November 2004, the UPOV Convention had 58 member countries. UPOV sets forth the minimum protection that the member countries should grant for the developers of new and distinct plant varieties (UPOV, 1991). Those minimum requirements are discussed below, in Section 24.3.1.

A specific form of *sui generis* protection is a plant patent, which is granted in the United States of America. A plant patent is different from a 'regular' utility patent. A unique feature of the protection system in the United States of America is that it provides two forms of *sui generis* protection (PVP protection and Plant Patent protection). The Plant Patent Act was enacted in 1930. A plant patent may be granted to new and distinct plant varieties that are invented or discovered, although excluding tuber-propagated plants and plants found in uncultivated areas. Plant patents are issued for 20 years from the date of filing.

24.2.2 Utility patent

Historically, a patent was a grant made by a sovereign that would allow for the monopoly of a particular industry, service or goods. Over time the concept has been refined from a public policy perspective and it has evolved to an agreement between the government and the inventor or creator.

In return for the right to exclude others from the practice of the invention, the government requests the inventor to fully disclose the enablement of the invention. Furthermore, the monopoly is limited by time, and clearly it is only applicable in the territory under the jurisdiction of the government granting the right.

In exchange for a limited-term right (usually 20 years) to exclude others from making, using or selling the invention, the inventor must provide a complete and accurate public description of the invention and the best mode of 'practising' it. This provides others with the ability to use that information to invent further, thus promoting technology development for the benefit of the society.

This right to exclude means that a patent is a 'negative right', since a patent holder may only exclude others from the using, manufacturing, copying or selling their invention during the lifetime of the patent right. Markedly, one can have a patent and

still have no right to practise the invention, for example due to lack of approval of some government instance. An example related to plant breeding is an inventor having a patent for transgenic plant in a country where genetically modified plants are not approved by the government.

Originally, utility patents were typically granted for various kinds of mechanical and chemical inventions. Along with the development of biotechnology rose the question of patentability of human-modified living organisms. A significant decision was made by the highest court in the United States of America in 1980 in Diamond v. Chakrabarty: a living artificially-engineered micro-organism was found to be patentable (Diamond v. Chakrabarty, 447 U.S. 303, 1980). The creation of a bacterium that is not found anywhere in nature constitutes a patentable 'manufacture' or 'composition of matter' as it is made by man.

Five years later the Board of Appeals and Interferences of the U.S. Patent and Trademark Office made a decision of patentability of a higher organism. In a case where genetically modified maize cell culture was sought to be patented, the Board held that sexually reproduced plants are eligible for patent protection (In Re Hibberd, 227 USPQ 433,185).

Today the international treaties set forth the frames for minimum protection of IP, but no treaty regulates how far a member country may extend the protection. Accordingly, there are variations among the countries as to what extent living organisms can be protected. The rulings of the United States of America courts, even if having effect only in the jurisdiction of the United States of America, have been important because they set a new tone into discussion of patentability of life forms everywhere in the world.

24.2.3. Copyrights

A copyright is a type of IP protection for 'authors' of original works. Basically, a copyright protects an original work and allows the author an exclusive right to reproduce the work, prepare derivatives of it, distribute copies of the work and perform the copyrighted work publicly.

Historically, copyrights have been important in protecting the rights of artists and authors. Today, copyrights are becoming more and more important in protecting the rights of database developers. In relation to plant breeding, copyrights may be a relevant means of protecting, for example, GIS databases supporting breeding, or databases containing gene sequences. Currently there are a number of projects that aim to sequence the genome of various crops; some of this information may be copyrighted.

The European Union (EU) provides an additional protection mechanism for databases: database protection can be sought in addition to regular copyright protection. Under the Directive on the Legal Protection of Databases, the database creators can protect unauthorized extraction and utilization of contents of their databases for a period of fifteen years from completion of the database. The Directive applies, however, only when the database creator is a citizen of an EU member country.

24.2.4. Trademarks

A trademark is a word, phrase, symbol, design or a combination of those, that distinguishes the source of one's goods or services from those of others, e.g. Kodak®. A trademark can be valid only when it is used in connection with the goods or services in commerce.

As in other industries, trademarks are also becoming increasingly important for the seed industry to brand its products. A

remarkable advantage of a trademark is that it is valid as long as it is in use in commerce. When the limited protection time of a patent or plant variety protection expires, a trademark can still be used to inform the customer of the specific qualities of the product. Outside of breeding industry, Kodak® is again a well known example: the patent right of the regular black and white film of Kodak expired about a hundred years ago, but still everybody knows exactly what they buy based on the strength of the trademark.

24.2.5 Geographical indications

Geographical indications are a kind of IP that has already been in use for rather a long time, but has been widely recognized as a means of protection only recently. A geographical indication is a sign used on goods that have a specific geographical origin and which possess qualities or a reputation that are linked to the place of the product's origin. Geographical indications serve as assurance of source or quality, and they are important in sense similar to a trademark.

In various countries, protection for geographical indications is provided under different concepts: geographical indications may be protected under laws against unfair competition, consumer protection laws, or laws for the protection of certification marks. In some countries there are special laws for the protection of geographical indications. In the United States of America, geographical indications are treated as trademarks.

Most commonly, a geographical indication consists of the name of the place of origin of the goods. Agricultural products typically have qualities that derive from their place of production and are influenced by specific local factors, such as climate or soil. Examples of geographical indications are 'Idaho' for potatoes from the state of Idaho or 'Roquefort' for the specific kind of French cheese.

The Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS Agreement) provides a high level of protection of geographical indications for wines and spirits. Currently, extension of this high level of protection to other products, such as agricultural products of developing countries, is under discussion in the World Trade Organization (WTO).

Note that geographical indications, akin to trademarks, do not protect the information embodied in the goods nor any method of producing or processing the goods. Rather geographical indications are rewarding groups of people that have developed a product, often over centuries of collective knowledge. Accordingly, geographical indications are considered as a part of wider policy to award protection for indigenous knowledge.

24.2.6 Trade secrets

Trade secrets are probably the oldest and the cheapest way to protect one's IP: having a trade secret simply requires as the term indicates, that the IP is kept secret. A trade secret could for example be a composition of a culture medium or a method to transform a plant species. A typical trade secret in the context of plant breeding is having the parent lines of a hybrid variety kept secret.

The positive aspect in trade secrets, in addition to its low cost, is that there is no expiration date. However, the negative side is that once the secret is out, the protection is gone and anyone is free to use the know-how.

24.3 PLANT BREEDERS' RIGHTS UNDER THE UPOV CONVENTION

As the UPOV Convention provides the framework for the most common and well

known of the *sui generis* systems for Plant Breeders' Rights we shall discuss the convention in more detail here.

The International Union for the Protection of New Varieties of Plants (UPOV) was established in 1961, and since then the provisions have been revised in 1972, 1978 and 1991. UPOV is a separate intergovernmental organization and is partially monitored by the World Intellectual Property Organization (WIPO). Currently UPOV has 58 Member countries, 25 of which are bound by the UPOV Convention of 1978, 31 by the Convention of 1991 and 2 by the Convention of 1961/1972. All the important agricultural producer countries are members of UPOV. More than half of the member countries are developing countries.

The goal of the convention is to provide an incentive to breeders to develop new varieties for the benefit of society by granting a limited monopoly to the breeders to commercialize new varieties. The Convention requires granting of protection for the varieties of all plant genera and species. New member countries of the Convention of 1991 must provide protection to at least 15 plant genera, and within ten years from joining UPOV they have to provide protection to all genera. The fact that a number of important countries, such as former Soviet Union countries, have joined UPOV only during the last years means that there is currently a situation where not necessarily all plant genera and species can be protected in these countries. Contrary to the requirement of all species being protectable, the Convention of 1978 requires protection of at least 24 species. An example of a 1978 Convention member is China, which became a member in 1999 and has currently a national list of protectable species containing 41 agricultural species. Even though the list covers the most important crop species, a large number of species cannot be protected in China.

PBR under the UPOV Convention provide the breeder of a distinct, uniform and stable variety with an exclusive right for a limited period for multiplication, offering for sale, selling, exporting, importing and stocking for these purposes. These breeders' rights do however not extend to acts done for non-commercial or experimental purposes, nor for purposes of breeding of new varieties. In other words, the UPOV Convention provides protection to distinct, uniform and stable varieties but also leaves certain exemptions for further breeding and non-commercial purposes.

24.3.1 Comparison of the UPOV Conventions of 1978 and 1991

Because most of the member countries are bound by the UPOV Conventions of 1978 or 1991, we briefly compare the minimum requirements set forth in these two conventions and discuss their implications.

Both of the Conventions require the variety to be distinct, uniform and stable (DUS) before protection can be granted. DUS-testing is mainly based on growing tests carried out by the competent authority of the member country where protection is sought. The Convention of 1991 additionally requires that the variety be novel, meaning that it has not been sold or commercially exploited for more than a year in member countries where an application has been filed, and not been sold in a non member country where an application for variety protection is filed for more than 4 years (for 6 years in case of trees and vines) before the application in the member country.

The Convention of 1978 protects commercial use of reproductive material of

the protected variety, while the Convention of 1991 protects varieties and products, including those that are essentially derived. The essential derivation provision is a similar concept to 'doctrine of equivalence' in the patent laws, aiming to prevent plagiarism. Essential derivation is a concept that has recently created a lot of discussion and therefore we shall return to it later in this chapter.

Of note is that UPOV 1978 restricts the countries where both patent and *sui generis* protection are available to grant only one type of protection for one and same botanical genus or species. The Convention of 1991, however, does not include this restriction. Accordingly, in the United States of America, which is a member of 1991 UPOV, the Supreme Court ruled in 2001 in J.E.M. AG Supply v. Pioneer Hi-Bred International that a plant breeder can obtain multiple protection for newly developed plant varieties; having a *sui generis* based variety protection does not exclude issuance of utility patent for the same if requirements for novelty, non-obviousness and usefulness are fulfilled as required for patents in the United States (J.E.M. AG Supply v. Pioneer Hi-Bred International, 122 S.Ct. 593, 2001).

The Convention of 1978 gives a 15-year protection from filing date for crops and 18 years for trees and vines. The most recent Convention, of 1991, grants 20 years of protection from filing date for crops and 25 years for trees and vines.

The Convention of 1978 grants so-called Breeders' Exemption, which means that breeders are allowed to use the protected material, without a licence, to breed new varieties. In the 1991 Convention, Breeders' Exemption is optional and it is up to the national government to implement legislation that respects Breeders' Exemption.

An essential and much discussed issue in UPOV is the concept of Farmers' Rights or Farmers' Privilege. Traditionally, farmers were free to save, re-use and sell harvested seeds. UPOV has brought some limitations to these rights. The Convention of 1978 did not include any specific requirements for Farmers' Privilege. This means that the farmers were left with the right to save and re-use harvested seeds of a protected variety. The United States of America implemented the Farmers' Rights of UPOV 1978, so that the farmer was allowed not only to save but also to sell the saved seeds, as long as the income from the saved seed was less than 50 percent of the total income of the farm. Now, as the United States of America is a member of 1991 UPOV, the farmer may no longer sell the saved seed, but does not need to pay royalties on re-used seeds. The European Union implements the 1991 Convention by allowing small-scale farmers to save and re-use seed without royalty payments, while re-use of seed of large-scale farmers is subject to reasonable royalties, which usually is 50 percent of regular royalty rate. Colombia, which is a member of Convention of 1978, but has rules mostly according to Convention of 1999, allows farmers having less than 5 ha to save seed (Louwaars *et al*., 2004).

24.4 INTERNATIONAL GOVERNANCE OF INTELLECTUAL PROPERTY

IPRs are based on national legislation and therefore the rights are usually territorial, so a patent is valid only in the country of the jurisdiction that granted it. However, during this era of globalization, there are several international treaties and conventions that are setting global frames for the IP legislation of the member countries. Below we review the treaties most relevant to plant breeding.

24.4.1 TRIPS Agreement

The World Trade Organization (WTO) originates from the GATT (General Agreement on Tariff and Trade) Uruguay Round negotiations during 1986 to 1994, and it is the main global instrument to support trade liberalization. Since 1994, WTO has gained a lot of influence and as of 13 October 2004 it had 148 member countries. The member countries are bound to several agreements covering goods, services and IP under the umbrella of WTO.

WTO administers the TRIPS Agreement of 1995. TRIPS attempts to harmonize the rules of IP protection of the member countries by establishing frames for minimum protection that each government has to provide to the IP of other WTO countries. WTO provides also a dispute settlement system for member countries having trade disputes over IP rights.

The basic concepts of the TRIPS Agreement are national treatment and most favoured nation (MFN) treatment. Accordingly, each member shall accord to the nationals of other members a treatment as favourable as it accords to its own nationals, and any advantage, favour, privilege or immunity granted by a member to the nationals of any other country in regard of IP protection shall also be accorded to the nationals of all other member countries. In simple terms, the member countries are bound to treat IP of any member country in an equal way.

The TRIPS Agreement builds on the Paris Convention for Protection of Intellectual Property of 1883, setting forth the patent system in the member countries. Similarly, the Berne Convention for the Protection of Literary and Artistic Works of 1886 is appreciated in setting forth the copyright system in the member countries. Both the Paris Convention and the Berne Convention are administered by WIPO, headquartered in Geneva, Switzerland.

Patent protection provisions of the TRIPS Agreement

The TRIPS Agreement describes the minimum rights that a patent owner must be provided in the member countries. Patent protection must be available for at least 20 years, which is the length of protection that almost every member country currently provides. Some countries, such as the United States of America and Australia, provide an extension of the 20-year protection for pharmaceutical inventions that need to be approved by other government agencies before the product can be offered in commerce. In the United States of America, human drug products, medical devices and food and colour additives, as well as animal drugs and veterinary biological products, are eligible for patent term extension. So far there are no similar extensions for utility or plant patent terms, even if, for example, transgenic plants need to be approved by other government agencies (in the United States of America by USDA, EPA or FDA) before they may be cultivated or offered for food or feed production.

Protection must be available for both products and processes in all fields of technology. However, the TRIPS Agreement has provisions giving governments a right to refuse to issue a patent for an invention if its commercial exploitation is prohibited for reasons of public order or morality. Also, the agreement allows governments to exclude from patentability diagnostic, therapeutic and surgical methods, plants and animals (other than micro-organisms), and biological processes for the production of plants or animals (other than microbiological processes).

Based on this TRIPS provision, many of

the member countries do not issue patents for plants. However, even if plant varieties may be excluded from patentability, in practise there still may be a way to get patent protection for plants: the European Patent Convention for example does not allow individual plant varieties to be patented, but the Board of Appeals of the European Patent Office ruled in 2000 in Novartis v. Plant Genetic Systems that genetically modified plants may be protected if the invention is not limited to a single variety (Novartis v. Plant Genetic Systems, G1/98 Transgenic Plant/Novartis II OJ EPO 2000). Here, clearly, the interpretation of the law provides patent protection to genetically modified plants. Canadian patent law excludes plants, as higher life forms, from patentability. However, in a recent case, the highest court in Canada found that growing transgenic plants containing a patented gene infringed a patent that claims the chimeric herbicide-resistance inducing gene and cells containing that gene (Monsanto Canada Inc. v. Schmeiser 2004 SCC 34). Therefore, even if Canadian law does not allow patenting of higher life forms, this decision implies that patent protection in Canada is extended to plants if a gene present in the plant's genome is claimed in the patent. The rational behind this is that by growing the plant that expresses a patented gene, one is using the patented invention.

Plant breeders' rights under the TRIPS Agreement

The TRIPS Agreement provides that:

> *members shall provide for the protection of plant varieties either by patents or by an effective sui generis system or by any combination thereof.* (Article 27.3 (b)).

The TRIPS agreement does not give any definitions for the term s*ui generis* but leaves us with the translation from Latin being 'specific' or 'of its own kind'. By giving no definition to this essential term means that member countries are left with 'free hands' to fashion their own protection system. The UPOV convention is one interpretation of what a *sui generis* system can be.

Another essential term not defined in the TRIPS Agreement's provision for protection of plants is the term 'effective'. How effective does an 'effective *sui generis* system' need to be? To clarify the meaning of the clause several countries are calling for further discussion on Article 27(3) of the TRIPS Agreement. Among others it has been proposed that the interpretation should extend the protection to traditional and indigenous knowledge. The discussion on Article 27(3) of the TRIPS Agreement is connected to the relationship of the TRIPS and the Convention on Biological Diversity (CBD), and is covered in Section 24.4.3.

WTO member countries have to have their IP laws in line with the TRIPS requirements. When the TRIPS Agreement came into effect in January 1995 it set out transitional periods for implementation for developed, developing and least-developed countries. Developed countries had to comply with TRIPS provisions by 1996, while the least-developed countries had until the beginning of 2006. Developing countries generally had until 2000 for the implementation, but the deadline was later postponed until 2005.

India is an example of a developing country that established its PVP legislation in order to comply with the TRIPS requirement. India enacted its plant variety protection laws in 2001. India has chosen *sui generis* legislation deviating from the norms set by UPOV (see Sahai 2003; Brahmi, Saxena and Dhillon, 2004). The effects of

the legislation remain to be seen after it is effectively implemented.

24.4.2 The Convention on Biological Diversity

The Convention on Biological Diversity (CBD) was established in 1992 as an outcome of the United Nations' Conference on Environment and Development (UNCED). Currently the CBD has 168 signatories. Of note is that the United States of America has signed but not ratified the Convention.

The main objectives of the CBD are to ensure conservation of biological diversity, to ensure the sustainable use of biological diversity, and to promote a fair and equitable sharing of the benefits arising from the utilization of genetic resources amongst member countries.

The CBD does not as such elaborate on IPRs, but it makes a clear statement on technology transfer as an important means to reach the goals of the Convention. Because much of the agricultural technology in the developed countries is protected by IPRs, the statement of technology transfer being an essential means to reach the goals of the Convention means that IPRs become an issue as well. Article 16.2 of the Convention states that

> *In the case of technology subject to patents and other intellectual property rights, such access and transfer shall be provided on terms which recognize and are consistent with the adequate and effective protection of intellectual property rights.*

Thereby, CBD clearly recognizes IPRs.

The Convention requires equitable sharing of benefits arising from commercial use of the biological resources and local knowledge of communities. The Convention also requires that access to genetic resources is subject to prior consent of the contracting party providing the recourses. These requirements have induced vast discussion and still unsolved questions of the compatibility of TRIPS and CBD.

24.4.3 Relationship of the TRIPS Agreement and CBD

CBD and the TRIPS Agreement approach the subject of IP protection from different perspectives: CBD has a focus on sustainable management of biodiversity, while TRIPS aims to provide a framework for adequate protection for IPR to reduce distortion of international trade. The relationship of the TRIPS Agreement and CBD has been widely debated in the TRIPS Council. A concern has been raised that implementation of the TRIPS Agreement may affect the ability of the WTO member countries to fulfill their CBD commitments.

Some developing countries have been arguing against granting patent rights for genetic material as is possible under TRIPS, because that might limit access to the resource and equitable benefit sharing, as required by CBD. Some countries have required that patent applications should be accompanied by disclosures regarding source of origin, any related traditional knowledge and evidence of equitable benefit sharing. Counter arguments have included notation that such requirements would limit availability of protection and this again would violate the principles of the TRIPS agreement. Furthermore, additional requirements probably would make the system expensive and complicated to implement.

Due to these concerns, several countries are calling for amendments to be made to the TRIPS Agreement to bring it into the line with CBD. At the same time, both the TRIPS Agreement and CBD are rather flexible in their language, and therefore the member countries have a lot of freedom

to find ways to implement both without conflict.

24.4.4 The International Treaty on Plant Genetic Resources for Food and Agriculture

The International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) was agreed in June 2004. It provides for a multilateral approach to access and to benefit-sharing of a selected list of plant genetic resources for food and agriculture. The list includes 35 crop genera and 29 forage species. *Ex situ* collections of these crops are held by the International Agricultural Research Centers (IACRs). The species in the list, even if not so many, provide about 80 percent of the world's food calories from plants.

The goals of the Treaty are very similar to the CBD, but ITPGRFA specifically addresses access to and fair sharing of the benefits generated from the commercial utilization of the genetic resources of the listed species in the food and agriculture industries. It thereby leaves utilization of the genetic resources in the pharmaceutical industry out of its scope, while CBD encompasses use of genetic resources in any field of technology. The central mechanism to implement the provisions for access and benefit sharing is a standard material transfer agreement (MTA).

The draft MTA attached to the ITPGRFA contains the language of Article 12.3(d) of the treaty, which has raised a lot of discussion. Article 12.3(d) states that

> *Recipients shall not claim any IP or other rights that limit the facilitated access to the plant genetic resources for food and agriculture, or their genetic parts or components, in the form received from the Multilateral System.*

The language of the article has been regarded as ambiguous as it is not clear whether, for example, isolated and purified compounds or gene sequences are patentable under this provision or not (Lettington, 2004). Currently parties to the treaty can interpret this provision rather freely, which means that the MTA may have different meanings in different countries, depending on the national legislation.

The ITPGRFA recognizes Farmers' Rights to freely access genetic resources, and to use and save seed. However, the implementation of Farmers' Rights is left fully to national governments. An implication of this is that the member countries of the treaty have to consider the relationship of Farmers' Rights to the already existing IP laws. The member countries might, for example, already have provisions for Farmers' Rights in their plant variety legislation. At the same time, member countries may end up protecting some aspects of Farmers' Rights through other legislation, such as laws regulating commerce in seeds.

24.5 CURRENT ISSUES IN IPRS AND PLANT BREEDING

24.5.1 Access to germplasm

Plant and animal breeding is different from any other field of technology in the sense that it is impossible to make progress in terms of inventions without having access to 'prior art'. A mechanic can invent something that provides a huge technical step forward without having the slightest idea of what is already out there. Opposite to this, a plant breeder can breed a better variety only by having access to germplasm. Despite this essential characteristic of the art of breeding, inventions related to plant breeding may still be protected by various forms of IPRs in a way similar to inventions in mechanics.

This is an issue that is raised time after time, because of concern that IPRs might prevent free access to germplasm and thereby affect the capacity to breed, research and provide better varieties for food and feed.

International treaties have provisions that are aimed to ease access to germplasm. As discussed above, ITPGRFA provides for *ex situ* collections of most important food and feed plants. The International Agricultural Research Centers (IARCs) of the Consultative Group on International Agricultural Research (CGIAR) hold over 600 000 accessions of crop, forage and agroforestry genetic resources. ITPGRFA requires a standardized MTA to guarantee that no IPRs shall be claimed for material received from the system. The goal of the treaty is to provide fair exchange of germplasm of the species included in the list.

Wild germplasm, in contrast, is an important part of the art of plant breeding, and wild germplasm might not be represented in genebanks alongside cultivation-based germplasm (Gepts, 2004). This argument would inevitably lead to a very broad and still unsolved issue of compatibility of existing plant IP system with the rights of indigenous people's traditional knowledge which issue has been recently discussed in Fingers and Shuler (2004).

24.5.2 Breeders' exemption in PBRs, and essential derivation

The 1978 UPOV Convention provides that a protected variety can be freely used as an initial source of variation for the purpose of creating other varieties, and that the breeder shall not be required to obtain authorization for marketing such varieties. This provision is known as Breeders' Exemption and it is a fundamentally important part of PVP. Breeders' Exemption guarantees that the germplasm sources remain accessible to the whole community of breeders. This also helps to keep the genetic basis for plant breeding as broad as possible and minimize the threshold for access to germplasm.

The language in the 1978 UPOV Convention has been interpreted to allow cosmetic modifications in breeding new varieties, such as inducement of mutations in ornamental plants. Development of methods for genetic engineering has brought further prospects of rapid modification of existing varieties. In order to prevent protection of new varieties with only minimal changes compared with the original variety without recognition of the breeder of the initial variety, the 1991 UPOV Act amended the concept of 'essential derivation'.

The core of the essential derivation concept is that the scope of the Breeders' Rights is extended to varieties that are essentially derived from the original breed. Essentially derived varieties may be obtained in various ways. The UPOV 1991 Convention gives a list of methods, including selection of natural or induced mutants, selection of a somaclonal variant, selection of variant individual plants in the initial variety, backcrossing and genetic engineering. Through this concept, if a breeder derives a variety essentially from another variety, such as inserting one new gene into the initial variety, the new variety can be protected if it is new, distinct, uniform and stable; but for as long as the initial variety is protected, the essentially derived variety cannot be exploited without authorization from the owner of the initial variety. In practice, this means that the breeder of an essentially derived variety would need a licence from the breeder of the original variety. If the essentially derived variety is derived from a public-sector-bred variety, there is naturally no need for a licence as the original variety was not protected.

The concept of essential derivation does not affect the right of a breeder to choose protected varieties for initial material. However, breeders clearly need to pay more attention to the results of their breeding work when the parents are protected varieties. If the new variety is too close to the protected parents it may be deemed to be essentially derived. The new variety may still be protected if it is distinct, stable and novel, but the breeder may need a licence before they can commercially exploit their essentially derived variety. The difficult question that remains is: "How close is too close?"

The UPOV Act does not provide any guidelines as to how the essential derivation is to be defined. The UPOV 1996 Annex provides that the dependency relations should be handled by the breeders themselves. Obviously, the first step is to define the essential characteristics of the species that is to be inspected further. The criteria for defining whether the characteristics are too close to the parent lines may be phenotypic or genotypic. The threshold determination for essential derivation should be done on a species-by-species basis, and currently there are various academic research programmes to evaluate the threshold values, for example by using separation distances of molecular markers as criteria. Lesser and Mutchler (2004) are of the opinion that the system where the status of the variety is to be worked out solely between the parties will not work, and that some oversight body must be involved to established consistent standards.

24.5.3 Research exemption in patent laws

Most of patent laws contain some kind of provision allowing experimental or research use of patented material or a method without a licence. The definition of experimental and research use may vary from country to country: what is experimental and therefore allowed in one country may not be that in another. Recently, Federal Circuit Court in the United States of America gave a very narrow definition for experimental use. In Madney v. Duke (207 F 3d. 1351 Fed Cir 2002) a university continued to use equipment patented by a professor that was no longer employed by the university. The university relied on its non-profit status and claimed use of the equipment being lawful under research exemption. The court ruled that the non-profit status of the organization is non-determinant and that the experimental use allows use of a patented method solely for amusement, to satisfy idle curiosity or for strictly philosophical inquiry. In Europe, the experimental use exemption seems to be interpreted less narrowly. The Supreme Court in Germany has ruled that clinical trials of a patented compound are non-infringing under the research exemption when the purpose was to find further information (Goddar, 2001).

Regardless of the different views of the United States of America and European courts, the breeder should still know whether the material (e.g. genes) or method they are working with is protected by patents. Even if they might not have thought they were breeding something that would one day become commercially exploitable, they might still be under an obligation to obtain permission from the owner to use the gene for research.

24.5.4 Freedom to operate in developing countries

IPRs are national, and therefore it is totally legal to use the material and methods in countries where the invention is not patented. As an example, various aspects in producing

GoldenRice, the vitamin A–rich transgenic rice, have been patented; a freedom to operate study showed that there are more than 70 patents related to the technology (Kryder, Kowalski and Krattiger, 2000). However, in most of the countries where rice is an important commodity, none or only a few of these patents were in force. In such countries, using or developing the technology further is legally completely correct. Issues may arise only when there is trade in the technology to countries where patents or other forms of protection are in force.

According to Pardey *et al.* (2003) there is still a substantial freedom to operate for most crops of major significance for food security in poor countries. Pardey argues that concern of freedom to conduct research by or on behalf of developing countries is seen as a way to draw attention away from real constraints. Real constraints according to the same authors are lack of investment in developing country research and lack of scientific skill to access modern technology, whether protected or not.

Koo, Nottenburg and Pardey (2004) show that from 2000 to 2002, 54 percent of the variety protection applications filed worldwide were filed in Europe or in the United States of America. The principal reason for the lack of filing activities in the developing countries is a lack of established protection means. At the same time, this data also indicates that a claim that IPRs are limiting the freedom to exploit plant-science-related inventions in the developing countries is an overstatement.

24.5.5 Farmers' Rights

Developing countries are required to introduce some form of plant variety protection under the TRIPS Agreement. However, as the TRIPS Agreement sets the frame very loosely, it remains for the countries to decide how the protection is to be implemented. Some developing countries have chosen to adhere to the UPOV Convention; others, such as India, are going to implement more liberal PVP.

The Farmers' Right provision of India's Plant Variety Protection and Farmers' Rights Act of 2001 has created a lot of discussion, because it seems to differ from anything that has been created under the UPOV Conventions. The Indian law allows farmers to save, use, sow, re-sow, exchange, share or sell the seed, providing that the farmer shall not sell the saved seeds in any packages or containers labelled in a manner indicating that the seeds are protected (Sahai, 2003). It has been argued that Farmers' Rights provisions as liberal as India's does protects only the brand of the breeder. In the Indian Act, there are also provisions for acknowledging the role of rural communities as contributors of landraces and farmers' varieties. A breeder wanting to breed an essentially derived variety needs to have permission of the communities (Sahai, 2003). The Act adopts all the suggestions of the UPOV 1991 Convention as to the methods that may be used to breed essentially derived varieties, in effect almost all the modern means of plant breeding. This leaves the Breeders' Exemption extremely narrow.

The International Association of Plant Breeders for the Protection of Plant Varieties (ASSINSEL) suggests that any national legislation authorizing farm-saved seed without reasonable limit and without safeguarding the legitimate interest of the breeders is not in conformity with the 1991 UPOV Convention. Additionally, ASSINSEL argues that any such legislation would be contrary to the meaning of the TRIPS Agreement, i.e. such a system would

not provide effective *sui generis* protection (ASSINSEL, no date).

The consequences of farm-saved seed to the breeder depend also on the contract that the breeder makes with the farmer. If the farmer pays royalties based on the amount of seed originally purchased, then farm-saved seed naturally reduces the earnings of the breeder. Another option for the breeder is to collect royalties as end-point royalties when the harvested crop is sold. By collecting end-point royalties, the breeder would benefit from the farm-saved seed provision provided that the farmer declares that the seeds they sell is of the protected variety. Contracts may also be used to oblige the farmer to keep a record of their practices. Such contracts would help the breeder to monitor the practice of the farmer in end-point royalty cases and would ease collecting royalties.

However, not only the implementation of the law in a country is important but the enforcement is as important, if not even more important. This of course means not only enforcement of Farmers' Rights, but also every aspect of the IP-laws of the country. Lack of enforcement of IP laws may lead to a situation such that of Argentina, where 25–50 percent of Roundup Ready® soybean seeds grown are from black market sources or saved by farmers from the previous year's crop (Robertson, 2000). Similarly Kowalski (2003) is worried about the future of agribiotech in China due to weak enforcement of the IP laws. Lack of enforcement leads to lower prices and eventually leads to unwillingness of companies to invest in countries having weak enforcement of IP laws (Giannakas, 2003). This author suggests that penalties determined under the TRIPS Agreement have to go beyond the norms of GATT, otherwise IPRs remain inefficiently enforced; simply offsetting the value of losses incurred by the innovator is not severe enough a punishment.

24.5.6 Other methods to protect unauthorized use of seeds

Contracts and MTAs

A breeder can control their rights over the material they own by contractual agreements, including MTAs, which are binding legal contracts between the technology provider and the receiver, and the most common legal documents controlling use of research material. The terms of MTAs can go far beyond the rights provided by a patent or other IP legislation. The MTA may include so-called reach-through clauses, whereby the technology provider may get rights to new varieties or other inventions and improvements that have been created by using the material provided through the MTA. The receiving party has to be clear as to what the implications are of signing an MTA before signing.

When selling seeds of a protected or a non-protected variety, a breeder may control the use by various kinds of contract. We discussed earlier how the breeder may control income by choosing the royalty basis defined in the contract with the farmer.

Two specific types of contract of significance in the seed industry are the so called shrink-wrap and brown-bag licences. Typically, a breeder includes in the seed package contractual language limiting the rights of the buyer. The seed bag may for example specify that the material inside the bag may be not be used for further breeding. By opening the package or by planting the seeds the user agrees with the contractual language on the package.

By these contractual means, the breeder can regulate the use of the material, even in countries without no IP legislation.

However, as both MTAs and brown bag licences are interpreted under the contract laws of the country, the enforceability of such means differs between countries.

Biological methods to control re-use of seeds

The modern technologies developed in the plant sciences provide certain methods to protect varieties from unauthorized use. These methods include hybridization and technologies usually called Genetic Use Restriction Technology (GURT).

Hybrid technologies were developed in 1930s and today hybrid varieties have been developed for most of the important cross-pollinated food and feed species. When hybrid seed is used for a second generation, part of the hybrid vigour is lost and therefore saving seed for re-use is not an optimal solution for a farmer. Rather the farmers are each year dependent on seed producer's new seed.

GURT is a biotechnology application of a system providing the breeder control over re-use of the seed. GURTs are not specifically developed for the purpose of enabling plant breeders to prevent re-use of the seed; rather the goal in developing the techniques have been for purposes such as preventing transgene escape into the environment. In any case, GURTs may also be a strong tool for preventing re-use of seed.

Currently it seems that there is quite a lot of discussion of the possible effects of this new technology in relation to farmers, research and the environment (e.g. Budd, 2004). Proponents of GURTS argue that GURTs provide seed companies and plant breeders with stronger control over plant varieties. This would enable greater cost recovery and provide more incentive for the plant breeding industry. GURTs would be a method to protect varieties in countries where only weak IP protection is available or where IP law enforcement is weak. The proponents also argue that transaction costs would be lower if there is no need for IP protection and therefore the benefit would come to consumers and farmers through reduced seed prices. Opponents of GURTs argue that GURTs will harm the farmers by taking away the ability to save and re-use seeds and will have adverse effects on food security and biodiversity.

The GURT technologies are still under development and in many countries genetically modified crops are in any case not yet in cultivation. Therefore there is no data that could prove either the fears of the opponents or the hopes of the proponents to be true. However, there are already indications that countries may not allow GURTs to be used in protectable varieties: the Indian Plant Variety and Farmers' Rights of 2001 does not allow the protectable varieties to include GURT technologies (Sahai, 2003).

24.6. PLANT BREEDERS' RIGHTS AND PARTICIPATORY BREEDING

Farmers have worked as plant breeders for centuries, but have in most cases not sought to protect their new materials through any form of statutory protection. Where a farmer can be identified as a breeder there would be no inherent problem in seeking protection, insofar as what is required for PBR is the identification of a 'breeder', and in the case of a patent an 'inventor'.

There are, however, a number of other elements to the current proprietary IPR protocols, in that farmers and communities are unable to meet some of the other statutory requirements as they relate to novelty, the time of the invention, prior sales, etc. There are also problems that in

many locations are associated with the inability to identify a breeder or inventor *per se* since the breeding activity is carried out at the community level—there is no longer a definable breeder or inventor.

A final issue that has been of concern in the literature has been the fact that indigenous materials are often not characterized or published. This may lead to perchance to another person breeding material with the same description, who would be able to gain protection on such material on the basis of claiming 'novelty' for the material in the absence of published information to the contrary.

Let us therefore address each of these issues and indicate arguments that are associated with each side of the issues.

24.6.1 Determination of the 'breeder' or 'inventor'

In modern crop improvement we are accustomed to several people being involved and collaborating on the inventive or breeding step. There would therefore be only limited problems with identification of the inventive steps involved and the persons involved, thus allowing for clear identification of breeders or inventors. In the same way that breeders then assign their material or invention to their institution, it is clearly feasible for a community or tribe to become the title holder to the invention or material bred or invented within that community. What cannot happen is that the community or tribe is deemed as inventor or breeder since they do not fulfil statutory requirements.

24.6.2 Novelty

In order for PBR or a patent to be granted there is a requirement of novelty (or distinctiveness). The challenge there is that the statutes tie caveats to the novelty concept, such as the time the product or invention has been 'in the market' or whether there is prior 'publication' of the information on the invention or material. Clearly, in community-based schemes this approach is hampered by the apparent informality of the system. This situation is clearly crucial to the area of landraces, where over many generations improvement have been made, but such improvements have become public goods basically because of time or sale of seed (even if informal).

24.6.3 Cost of filing

Clearly, in some cases, there are severe constraints on small communities because of the cost of filing applications. This, however, is no different from the problem that faces individual inventors who want to patent an invention. The crucial question to ask here is 'why' the person or community wants to seek protection. If the goal is to licence the materials for income, the answer is to spend the money and seek the protection. If the goal is to prevent appropriation by others of the intellectual knowledge, then simply publish the data in written form, take your credit, and while others can use the invention, they cannot gain any exclusivity or proprietary protection for the material.

24.6.4 Decisions are needed

From the above it follows that what is really needed are educational steps and clear understanding as to the goals and aims of those communities that are improving germplasm. In short, what is it that the communities are seeking? If the key is recognition of input and prevention of proprietary exploitation by others, then publication of the data serves a vital purpose. There are no doubt international agencies that are willing to provide funding,

either to communities to protect their new assets, or to assist with documentation and publication.

24.7 CONCLUSIONS

The plant breeding industry has encountered changes during the last decades. New breeding methods have raised issues of IP protection. The menu of the means that a breeder may use to protect their invention is not limited to PVP but includes several other means as well. In addition to various forms of IP, breeders may also use other legal forms to control use of varieties.

There is an increasing amount of legislation and international treaties regulating issues related to IP in plant breeding. Nevertheless, a lot of the decision-making is still left to national governments.

There is certainly no one correct and acceptable system to be implemented in each and every country to provide reasonable rights for farmers, breeders and industry. The international treaties set frames for minimal protection. The individual countries are left with rather a free hand in tailoring their national IP laws.

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CHAPTER 25

The impact of participatory plant breeding

Jacqueline A. Ashby

25.1 INTRODUCTION

Understanding how and when participatory plant breeding (PPB) is a proven complement to non-participatory breeding approaches builds on almost three decades of practical experience with PPB, but also relies on a growing body of impact-assessment research. PPB is a strategy with its own set of methodologies for plant breeding that applies in situations where the demands of producers, traders, industries and consumers for varietal traits are poorly understood and difficult to diagnose with conventional market research methods. This occurs where there is a high degree of risk and uncertainty due, for example, to volatile markets, climate change or very diverse agro-ecologies in the growing environment. PPB may also apply when producers and other stakeholders in a value chain, or even society at large, want to exert a high degree of control over decisions about the use of plant genetic resources and the kinds of plants that are introduced into the food system. The *impact* of PPB refers to the long-run effects of using its strategies, methodologies and tools. As a set of methodologies, PPB influences the agricultural extension and research process, as well as the productivity and welfare of producers, traders and consumers of the end products of PPB.

This chapter lays out the theory of change underpinning the impact of PPB and the evidence of impact from studies and reports of PPB programmes, the majority of which are located in developing countries. The theory of change is an explanation of the causal relationships that link the results of PPB to its impacts. To begin, a short review of definitions of participation is essential because different modes or types of participation in plant breeding can produce different impacts. Once the implications of different modes of participation for impact are clear, then hypotheses and evidence about the impact of PPB can be classified and analysed. This chapter lays out the hypotheses contained in the PPB theory of change in the framework of the impact pathway, a tool for showing the cause and effect linkages among different categories of impacts. Key issues related to research design and the analysis of cause and effect are reviewed, because these influence the extent to which we can confidently attribute certain impacts to PPB. Finally, the chapter examines the evidence that can be brought to bear on the principal components of the PPB impact pathways, using examples to illustrate findings obtained from over twenty years of experience with PPB in crop improvement programmes in more than 15 countries around the world (Walker, 2006; Ashby & Lilja, 2004; Vernooy, 2003). The impact of using PPB is multifaceted and includes changes in the research process as well as in knowledge, technology design and social organization. In developing countries, where markets are inefficient and it is difficult to discern the demand of small-scale farmers for new plant varieties, agricultural researchers use PPB to obtain feedback about farmers' varietal preferences. PPB can enable breeders to incorporate farmer knowledge into breeding strategies, objectives and methodologies: this knowledge refers to local environments, indigenous plant genetic resources, and local organizational capacity for participation in PPB. It can also enable farmers to incorporate advanced scientific knowledge into local practices, such as their customary, back-garden experimentation with plant varieties or seed banks. PPB changes the way the breeding process is organized and its costs when it increases cooperation between breeders and farmers in research.

Key impacts of PPB are to produce plant varieties that are well tailored to poor producers' needs, to shorten the amount of time plant breeding programmes need to get appropriate materials into farmers' fields and so accelerate adoption and seed dissemination. This is an impact on research efficiency related to improving the rate of innovation overall. In some situations, PPB helps to maintain or increase plant genetic diversity in farmers' fields and improves agricultural sustainability. PPB carried out with farmer groups improves farmers' organizational and social capital, as well as individual farmers' knowledge and skills and capacity to learn and experiment: all contribute to more resilient and sustainable farming systems. In addition, PPB is expected to have welfare impacts by increasing poor farmers' access to improved varieties, their productivity, nutrition, marketing and incomes. Given the important role played by women in managing plant genetic resources in many farming communities, PPB can affect gender equity.

PPB has evolved mainly to address the difficulties of poor farmers in developing countries. Widely seen as having advantages for use in low yield potential, high stress environments, PPB is most often applied when specific adaptation is sought. For this reason, a review of plant breeding methodologies in the CGIAR recommended in 2001 that it should form an "organic part of each Center's breeding program"(TAC, 2001: 24). However, some practitioners have results showing that both specific and wide adaptation are possible (see for example, Joshi, Staphit and Witcombe, 2001).

In industrialized agriculture, where wide adaptation is prized and markets drive demand for research, PPB maybe less useful from a research efficiency perspective, although farmers' local knowledge has on occasions proved a vital resource for developing new crop varieties (e.g. Walker, 2006). Nonetheless, in emerging markets, such as organic agriculture, PPB can have advantages. For example, in France, formally including producers in PPB for organic agriculture has proved useful for determining breeding objectives and methodology (Chiffoleau and Desclaux, 2006). This experience illustrates how PPB may prove useful in the debate about the welfare impacts of plant breeding in view of consumer scepticism about genetically-modified (GM) crops, and concern about how plant breeding affects food safety. PPB can promote informed participation and trust in research among consumers and producers.

In summary, impacts of PPB in international crop improvement research are associated with improving research relevance and efficiency via feedback from farmers, traders and consumers, and the welfare impacts of a faster and more relevant supply of new plant varieties to small-scale producers. There is, in addition, the issue of the impact of PPB on the costs of research and innovation. This is a complex issue, which is still relatively under-researched, but for which there is some evidence, discussed later. PPB may increase research costs compared to experiment-station-centred breeding because it is typically decentralized and requires work at multiple sites. At the same time, after 2–3 years of cooperation with a PPB programme, farmers increase their capacity to manage varietal evaluations and trial plots independently, and may assume some of the costs of adaptive research. In this situation, a criticism of PPB is that overall breeding programme costs are reduced but farmers' costs go up. However, the benefits of PPB to farmers include a

reduction in the risk of productivity and income losses from planting ill-adapted, poorly performing varieties in their fields (a common experience for poor farmers receiving experiment-station-centred recommendations). Moreover, if PPB places improved varieties and seed in farmers' fields more quickly than other approaches, then farmers' income stream from new varieties will increase sooner. These gains must be factored into the overall cost–benefit assessment of PPB.

Before we can draw conclusions about the impact of PPB it is essential to make some important distinctions among the different types of participation used in plant breeding, and that is the topic of the next section.

25.2 WHAT DOES 'PARTICIPATION' MEAN IN PARTICIPATORY PLANT BREEDING?

The term "participatory plant breeding" (PPB) is used in this discussion to refer to the entire process of setting breeding objectives, making crosses, developing finished varieties and their release up to and including the supply of basic seed to growers. For the purposes of impact assessment, PPB refers to the full spectrum of breeding activities, including participatory varietal selection (PVS), much in the same way that trials evaluating finished varieties are generally understood to form part of a breeding programme. Some PPB specialists distinguish PPB as a breeding programme that includes farmers making crosses, as distinct from one in which breeders use farmers' suggestions or preferred local varieties to make their own crosses. They use the term PVS to refer exclusively to the participation of farmers in the evaluation of finished varieties and have demonstrated that PVS is a rapid way of identifying farmers' preferred cultivars. PPB can then use as parents cultivars identified by PVS (see, for example, Witcombe, Joshi and Staphit, 1996; Witcombe *et al*., 2005). In practice, PPB is a continuum of practices and differences from PVS are not rigid (Morris and Bellon, 2004). Several programmes use a mixture of approaches, often because practice evolves over time as breeders and the participating farmers learn how PPB works.

Discussion of the impacts of PPB requires a clear definition of what is meant by 'participation', because this term is loosely applied to a diversity of approaches for involving farmers in plant breeding, and various types of participation have different impacts. Participation refers to the relationship between producers and breeders, well recognized as a critical factor in many successful breeding programmes (Walker, 2006). One dimension of this relationship can be defined by the use of a functional or an empowering approach to participation. Functional and empowering approaches to PPB can be thought of as opposite ends of a continuum in the degree of participant empowerment. Functional participation in plant breeding improves research efficiency by involving prospective users of the results (farmers, intermediaries, traders, industries and consumers) in prioritizing and evaluating traits important to them, such as plant architecture, market appeal, storage and cooking quality. Functional approaches tend to leave the balance of power in decision-making in the breeding process essentially unchanged, i.e. plant breeders (and their employers) make most of the critically important decisions. Empowering participation changes the balance of power in decision-making in the breeding programme, usually in favour of giving non-research interest groups a more important role in key decisions about

the end product, as well as in how the research is carried out. An empowering approach to farmer participation frequently alters breeding objectives and procedures, including the environments and cultural practices used to screen varieties. This leads to different results, as discussed in more detail below (Okali, Sumberg and Farrington, 1994; Ashby, 1996).

A similar distinction is made between PPB carried out in formal plant breeding research programmes versus farmer-led programmes. Farmer-led PPB is typically run by NGOs, and in several cases involves extensive research, but has different objectives from public sector crop improvement research. These may include community empowerment, biodiversity conservation, disaster relief or skills development (McGuire, Manicad and Louise, 2003). By definition, farmer-led programmes aim to empower farmers, but in practice can employ as varied or narrow a mix of types of empowering and functional approaches to participation as formal breeding programmes.

A more important distinction is between participatory research and participatory learning. Participatory *research* in agriculture is conducted to investigate questions for which neither scientists nor producers have an agreed explanation. Like all research, it involves risk and uncertainty about the outcomes of experimental treatments and it combines use of scientific method with native empiricism. The result is new knowledge, usually a blend of scientific and indigenous. The impact of PPB on this co-production of new knowledge may increase as the level of farmer-scientist cooperation and farmer empowerment increases. In contrast, participatory *learning* uses principles of discovery learning to promote sharing of established knowledge. Adult education in particular uses discovery learning because adults learn better when they uncover concepts and facts themselves than when they are told about them. Especially in agriculture, discovery learning involves farmers in running on-farm experiments very similar to the varietal trials used in participatory breeding. The key difference is that the participatory learning facilitator knows ahead of time what the experiments will show and, indeed, has designed the experiments to demonstrate a known practice or principle. Because participatory learning for agriculture uses experiments, it is easily confused with participatory research. PPB demonstration trials use participatory learning to share existing knowledge about varieties. PPB research experiments combine farmers' and breeders' ideas to test jointly conceived hypotheses and co-produce knowledge that is new to all concerned. Indeed, the performance of varieties grown on small farms in marginal environments is often so unpredictable that programmes starting out with a participatory learning focus find themselves drawn inexorably into participatory research, because their varietal demonstration trials did not produce the results expected.

Participation in plant breeding research (and in research generally) is based on the principle that participation of end users in the co-production of knowledge generates a higher level of understanding, ownership and trust in the information, and increases their capacity and willingness to make use of it. All actors involved in PPB research, including the scientists, have hypotheses but no *a priori* certainty of what results will be obtained. The experimental process is undertaken in conditions of mutual uncertainty and shared risk. PPB research typically involves cooperation between

farmers and scientists in one or all of the following: establishing breeding objectives; identifying desirable traits so as to design plant ideotypes; selection of parents; selection in early generations; and screening of advanced lines. Scientists and farmers bring very different kinds of complementary knowledge and expertise to PPB research, but they have a common goal of testing hypotheses to answer questions to which neither know the definitive answer. Plant breeding programmes also use participatory learning to demonstrate finished varieties. A different use of participatory learning is when PPB programmes seek to improve farmers' capacity to participate in research in an informed manner, as when farmers are taught basic principles of heritability, techniques for making crosses or how to keep trial records.

In practice, PPB programmes often use a combination of both participatory research and participatory learning at different stages in the plant breeding process. For example, in the PPB methodology called 'Mother-Baby Trial' the Mother varietal trial is a researcher-designed and researcher-managed experiment. This trial is a platform for demonstration and participatory learning by farmers about the varieties that breeders are testing. Farmers then select those varieties they want to try out on their own farms. The farmer-designed and farmer-managed Baby varietal trials are a platform for participatory learning by breeders about farmers' criteria for varietal selection and management. Joint farmer-breeder participation in research and the co-production of new knowledge occurs when the combination of results and recommendations from Mother and Baby trials are made jointly. However, if breeders interpret data, draw conclusions and make recommendations from Mother-Baby trials independently of farmers, then farmers' are limited to a participatory learning role. The important question is: does this difference affect the recommendations and the eventual impact of PPB? The chapter will return to this question when evidence of PPB impact is analysed.

Different types of participation

PPB impacts are likely to vary depending on the type of participation used and whether or not the primary objective of participation is the co-production of new knowledge. The objectives are what differentiate approaches, not the methodologies or tools they use for facilitating participation, whether these involve participatory rapid appraisal (PRA), Mother-Baby trial, farmer field schools (FFS), farmer research committees (CIALs), participatory technology development (PTD) or others (Johnson, Lilja and Ashby, 2003). The key distinctions are:

- whether participation promotes or excludes the co-production of new knowledge between farmers and scientists ; and
- the timing of farmer participation: specifically, how early does participation occur in the breeding cycle?

Lilja and Ashby (1999) constructed a typology for empirical analysis of participation based on the principle that the way decisions are shared at different stages of a plant breeding process will structure the opportunities for co-production of new knowledge. The typology defines two groups of decision-makers: 'scientists' who include research programmes and extension agencies; and 'farmers' who include all the intended users of the PPB varieties such as consumers, traders and processors. These are ideal types of participation along a continuum in which 'farmers' are progressively more empowered, from

conventional, in which there is no 'farmer' empowerment, to farmer experimentation, in which there is no 'scientist' empowerment. Different probabilities of co-production of knowledge are embedded in the typology: the most equitable balance of power in shared decision-making and the highest likelihood of shared knowledge generation is defined by the 'collaborative' type. Consultative and collegial participation both decrease the probability of co-production of knowledge.

The five types of participation are as follows:

- ***Conventional*** (no farmer participation). 'Scientists' make the decisions alone without organized communication with 'farmers'.
- ***Consultative.*** Scientists make the decisions alone, but with organized communication with farmers. Scientists know about farmers' opinions, varietal preferences and priorities through systematic one-way communication with them. Scientists may or may not factor this information into their decisions. Decisions are not made with farmers nor delegated to them.
- ***Collaborative.*** Decision-making authority is shared between farmers and scientists based on organized communication between the two groups. Scientists and farmers know about each other's ideas, hypotheses and priorities for the research through organized two-way communication. Plant breeding decisions are made jointly, neither scientists nor farmers make them on their own. Neither party has the right to revoke or override the joint decision.
- ***Collegial.*** Farmers make plant breeding decisions collectively, either in a group process or through individual farmers who are in organized communication with scientists. Farmers obtain information about scientists' priorities and research hypotheses through organized communication between the two groups. Farmers may or may not let this information influence their decisions.
- ***Farmer experimentation*** (no scientist participation). Farmers make the decisions either in a group or as individuals on how to experiment with and introduce new genetic material without organized communication with scientists.

The effect of any one type or combination of types of participation on the probability of co-production of new knowledge and the eventual impacts of PPB depend on how *early* in the breeding process farmer participation is sought (Joshi, Staphit and Witcombe, 2001; Lilja and Aw-Hasaan, 2002). Timing affects the objective and impact of the participation, and, in particular, the likelihood of co-production of new knowledge. To illustrate this point, consider the possible outcomes of one type of participation, collaborative participation, at three different stages of a PPB process: the early planning and design stage, the intermediate testing stage when fixed lines are evaluated, and the final diffusion stage, when seed is multiplied and distributed. The outcomes of collaborative participation will vary depending on the stage in the breeding process at which it is used. Collaborative participation in the early, design stage of PPB enables farmers to contribute genetic materials and actively engage in planning crosses: they can influence overall breeding priorities. Novel parents and crosses often result, affecting the variability on which subsequent stages of the PPB programme will build. In contrast, collaborative participation in the later, testing stage of the breeding process involves farmers in evaluating fixed lines: as a result, the varieties produced and impacts will be

different from those developed with farmers involved at the design stage. Collaborative participation at the late, diffusion stage of PPB means farmers can only influence when, where and with whom varieties are demonstrated and multiplied for seed, but not the kinds of varieties available to them.

Early participation that enables farmers to help set breeding goals has the collateral effect of encouraging farmers to engage more actively with the breeding programme and adopt more rapidly. For example, in a community meeting, Nepali women farmers asked for quality improvements in a cold-tolerant rice variety. Farmers and breeders managed and screened jointly from F_5 bulk families and the resultant were superior to the best entries from the conventional breeding programme. Released by the national programme, the new variety spread rapidly to over 30 percent of rice area in the participating villages (Staphit and Subedi, 2000). Witcombe and Virk (2001) argue, based on a number of studies, that when a breeding programme based on a few crosses, the choice of parents is crucial and that farmer participation is highly effective in narrowing this choice made at the early stage in the breeding process. A methodological study of PPB using fixed lines and segregating populations found that farmers used a higher selection pressure than breeders, selecting about half the number of lines on station and about one tenth of the number of lines on farm compared to breeders. Entries selected by farmers yielded as much as those selected by breeders (Ceccarelli *et al*., 2000). This substantial body of research demonstrates the value of integrating farmers' intimate knowledge of their production environments into key breeding decisions.

In the typology described above, Lilja and Ashby (1999) divide the innovation process into three stages: design, testing and diffusion. In PPB (Weltzein *et al.*, 2003) these stages roughly correspond to:

- ***Design***. Setting breeding goals and generating variability. Decisions are made about basic parameters of variety type(s), preferences, and user needs. In most programmes, this stage involves designing and making crosses between diverse parents with complementary trait combinations. It may involve building base populations for cross-pollinating crops or the generation of new progenies for testing.
- ***Testing.*** In plant breeding, decisions are made about how to narrow down the new variability achieved in the design stage from several thousand to a few hundred progenies or clones (in the case of vegetatively propagated crops), and includes selection in segregating generations in self-pollinated crops. In population improvement schemes this is the progeny testing stage. In plant breeding this stage includes the testing of experimental materials on-station and, increasingly, on-farm. This testing looks for desired productivity traits, adaptation and acceptability, usually in replicated plots over a range of locations with increasing plot sizes. Testing continues until varieties are proposed for release.
- ***Diffusion***. This includes varietal release, demonstration under farmer management on farms, and the identification of a seed production and distribution system. Although this stage goes beyond the purely technical breeding process, the seed system may present a bottleneck to eventual impact, especially in poor countries, that needs to be taken into consideration early in the design stage.

Lilja and Ashby (1999, 2007) constructed a matrix in which any one of the five types of participation described earlier can be used

in one or more of the three stages of PPB. With data obtained by using this matrix for interviews with 49 PPB programmes and projects about 32 key decisions in the design and testing stages of PPB, multiple correspondence analysis (MCA) was applied to identify types of participation used in PPB. MCA identifies relatively homogenous groups of cases based on selected characteristics, in this case patterns among PPB programmes in the way they use different types of participation at different stages of the PPB process. The results showed that these PPB programmes fall into different clusters based on their participation practice. The cluster with the largest number of PPB projects (61 percent) adheres mainly to collaborative participation.

25.3 IMPACT PATHWAYS FOR DIFFERENT TYPES OF PARTICIPATION

Impact pathways provide a framework for systematically mapping the cause-effect relationships (in the form of a flow chart), whereby a given intervention leads to a set of impacts, either expected or observed (Douthwaite *et al.*, 2003). This section uses the impact pathway framework to compare impact pathways for PPB and PVS, their use of various types of participation and differences in their impacts. Impact pathways can be diagrammed as flow charts, showing products of varietal selection (Figure 25.1, PVS) or making crosses (Figure 25.2, PPB) leading to outputs and finally to impacts. The impact pathway is a tool that a breeding programme can use to clarify its expected or actual outcomes and impacts: for those shown in Figures 25.1 and 25.2 the topmost pathways are generic but they can be changed to reflect specific programme goals.

Products in an impact pathway refer to results over which programmes have a high degree of control and a high probability of achieving. Outcomes in an impact pathway refer to the effects of using the products in the short term (usually about 2 or 3 years).

FIGURE 25.1
Impact pathways for PVS

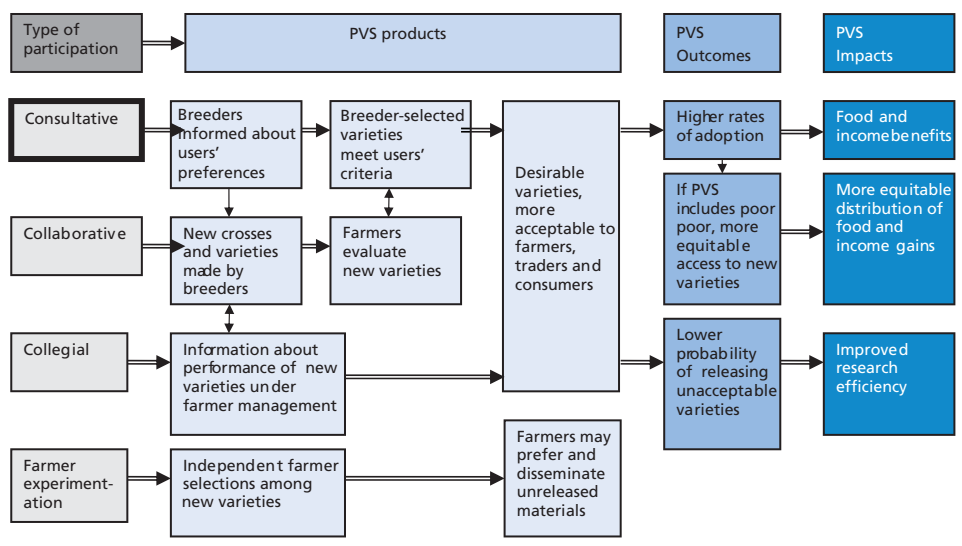
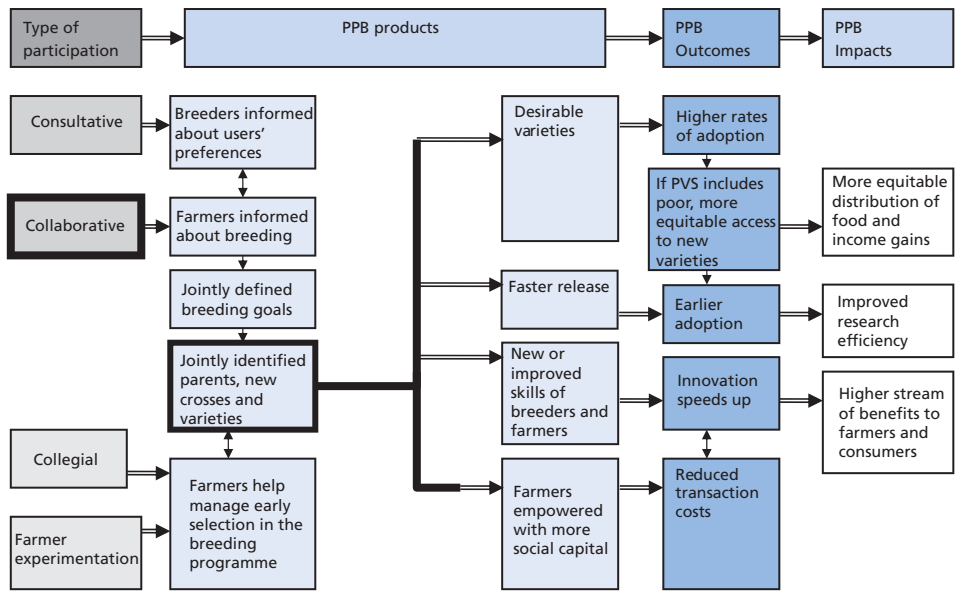


FIGURE 25.2
Impact pathways for PPB



Impacts refer to effects that take more time to achieve. In the category of PVS products, Figure 25.1 shows that consultative participation produces information about farmers' varietal preferences, which breeders then use to identify existing varieties or cultivars that are new and more desirable to the target population of farmers. If existing varieties are not suitable, breeders use information about farmer preferences to produce new varieties that are typically evaluated with farmers. This may involve collaborative participation, in which farmers are involved in decisions about which varieties are advanced or released. Typically, farmers engage in some of their own experimentation, either guided by the breeding programme (as in the Mother-baby trial approach, for example) or independently with escapes from formal trials. The end product of PVS is varieties that are more desirable to producers (and usually also to traders and consumers). The outcome is that more farmers adopt PVS varieties over wider areas, leading to increased food and income benefits. Another impact is increased research efficiency due to more relevant and desirable research products.

The impact pathway for PPB in Figure 25.2 illustrates how impacts change as participation occurs at earlier stages in the breeding process. As in PVS, a product of PPB is information about farmers' varietal preferences. However, in PPB, this exchange takes place early enough for breeding objectives to be defined jointly. In some PPB programmes, parents are also identified and crosses jointly planned. Thus, PPB involves reciprocal learning by farmers of key information about breeding strategies and some basic procedures. Farmers help manage early selection in the breeding programme and this activity harnesses a lot of the energy and resources that farmers

otherwise expend on trying new varieties on their own. In addition to more desirable varieties, PPB characteristically produces varietal releases more quickly, reducing the time from first crosses to release by as much as 30 percent. Two other impacts of PPB are, first, the increased skills and knowledge for both farmers and breeders of 'how to' collaborate to co-produce improved crop varieties—this results from collaborative participation early in the breeding process. Second, norms of trust and reciprocity (social capital) developed between breeders and farmers who collaborate, as well as among groups of farmers, lead to observable increases in farmers' self-confidence and leadership (empowerment). One outcome is to reduce the transaction costs for numerous actors involved in developing, releasing and disseminating new varieties, which has a positive effect on the overall speed of innovation in the agricultural R&D system. Increasing the speed not only for making a given variety available to growers but also for the whole process of introducing new varieties, thus dramatically increases the benefit stream.

Evidence of impact

This section draws on a wide range of reported case studies of PPB, both published and unpublished. Fifty cases were included in a survey conducted by the CGIAR Participatory Research and Gender Analysis (PRGA) Program to obtain expert opinion from over 150 participatory research practitioners and form part of the PRGA's inventory of cases .This information is publicly available on the Program's Web site (Ashby and Lilja, 2004). Between 1987 and 2007 the PRGA made a systematic effort to stimulate impact assessment studies, synthesize their findings and promote their publication in peer-reviewed journals. The availability of published studies on the impact of PPB has increased notably in the past five years, including work done by the World Bank for the 2008 World Development Report (Walker, 2006).

Caution is required in using many of the available studies of PPB to make inferences about its impacts. In an impact pathway, outcomes are more difficult to predict or achieve than products, and impacts are even more difficult because of the passage of time and the numerous intervening factors that may change what happens. As one moves across an impact pathway from products to impacts, it is usually increasingly difficult to determine cause and effect. For example, whether higher returns to research on new varieties can be attributed to PPB, to the other types of research involved, or to market or policy changes that stimulate farmer adoption. One approach to this problem of attribution is to design impact assessment studies to include a counterfactual that permits comparison of 'with' and 'without' effects and, in some instances, also comparison of conditions before and after the intervention being assessed. However, most studies of PPB were not designed to provide a formal impact assessment. Although ideally we would compare PPB programmes with breeding programmes that did not use PPB, this is seldom possible. Another difficulty is selection bias, an issue in any analysis where the treatment groups are not randomly selected. PPB programmes may choose to work with specific farmers or communities in a way that biases the observed impacts. For example, they may work with more educated farmers, more organized farmers or more wealthy ones. Then impacts attributed to PPB may in fact be due to farmer education, organization or wealth. Finally, PPB efforts that fail to produce desirable varieties, or any of the

other PPB products noted in Figure 25.1, may be under-reported in the literature, so that we tend to have more evidence about success than about failure.

Some PPB impacts are relatively easy to measure using established impact-assessment methodology. Agronomic and economic outcomes can be assessed at the farm level by measuring yield changes, net income over time and externalities such as changes in pest pressure or soil loss. Increases or decreases in costs are also straightforward. However, when empowering participation is used, part of the effects of PPB is on productivity and in particular on accelerating innovation in varietal improvement These impacts that are external to the technology are often referred to as disembodied effects, and pose a greater challenge for impact assessment as they are more difficult to quantify (Lilja and Dixon, 2008).

Impact pathway: PPB and PVS produce more desirable varieties leading to higher rates of adoption

Numerous studies conclude that PPB and PVS improve the acceptability of bred varieties to poor farmers in difficult environments by including their preferences in criteria for developing, testing and release. Small-scale farmers often rank varieties in order of preference differently from breeders. Many examples are available that show how PPB clarifies where there is agreement between breeders and farmers on desirable traits and where they disagree: cassava in Brazil and Colombia (Iglesias, Hernández-R and López, 1990); Hernández, 1993; Fukuda and Saad, 2001); pearl millet in Namibia (Ipinge, Lechner and Monyo, 1996; Monyo *et al.*, 1997a,b) and in India (Weltzein, 2000); maize in Mali (Kamara, Defoer and De Groote, 1996; Defoer, Kamara and De Groote, 1997); beans in Colombia (Ashby, Quiros and Rivers, 1989; Ashby, 1986; Kornegay, Beltrán and Ashby, 1996), in United Republic of Tanzania (Butler *et al.*, 1995), in Ethiopia (Mekbib, 1997), and in Rwanda (Sperling and Scheidegger, 1996; Sperling, Loevinsohn and Ntabomvura, 1993); tree species in Burundi (Franzel, Hitimana and Ekow, 1995); potatoes in Rwanda (Haugerud and Collinson, 1990), in Bolivia (Thiele *et al.*, 1997; Gabriel *et al.,* 2006), in Peru (Ortiz *et al.,* 2004), and in Ecuador (Andrade and Cuesta, 1997); rainfed rice in India (Maurya, Bottrall and Farrington, 1988); rice in Bangladesh, India and Nepal (Joshi and Witcombe, 2003; Joshi *et al.*, 2008), and in East India (Cortois *et al.,* 2001); maize in India (Virk *et al.*, 2003, 2005), in Ethiopia (Negasa, 1991), in Honduras (Gómez and Smith, 1996), and in Brazil (Machado and Fernandes, 2001); and barley in the Syrian Arab Republic, Morocco and Tunisia (Ceccarelli *et al.,* 2001a, 2003). A careful study in Mexico (Bellon *et al.*, 2000) was designed to select a subset of 17 populations for PPB from a set of 152 maize landraces. The suggestions of men and women in farm communities, professional plant breeders, gene bank managers and social scientists were obtained. The results showed that when germplasm choice did not include farmers' ideas, traits and materials important to farm households were often overlooked. The involvement of women farmers in the participatory development of maize seed systems in China resulted in a broadened national maize genetic base and improved maize yields (Song, 1998). This experience, by now so diverse with respect to crops, cultures and production environments, demonstrates the efficacy of participatory selection in producing varieties for poor farmers who are otherwise excluded by conventional crop improvement programmes.

A rigorous study conducted by the ICARDA barley breeding programme compared the number of high-yielding varieties obtained (termed selection efficiency) using different approaches. The breeder was more successful than farmers in selecting on station under high rainfall conditions, but farmers were more successful under stress conditions. A t-test of significant difference showed that farmers' selections were as high yielding as breeders' selections (Ceccarelli *et al.*, 2001a). Subsequently, the same programme conducted an important set of experiments on farmer participation in barley breeding in Morocco, the Syrian Arab Republic and Tunisia, where barley is the main crop for poor farmers in marginal, rainfed areas. Breeders' trials were planted both on research stations and in farmers' fields. Selection was done independently by professional breeders and farmers and data were gathered on their selection criteria and selection efficiency. Farmers used selection criteria not normally used by breeders because of the importance of the crop as source of animal feed. Disease, a major selection criterion used by breeders, was almost entirely neglected by farmers. Farmers successfully selected some of the highest yielding lines in their own fields and also on station. (Ceccarelli *et al*., 2001b).

By successfully understanding and incorporating farmers' criteria for acceptability, PPB consistently enables breeding programmes to 'break through' adoption bottlenecks. In Ethiopia, for example, over 122 varieties of cereals, legumes, crops and vegetables were released, but only 12 varieties had been adopted by farmers, prompting a start with PPB (Mekbib, 1997). In Brazil, the national agricultural research institute, EMBRAPA, confronted years of non-adoption of new cassava clones. Once PPB was implemented several clones were released which were highly acceptable to farmers (Fukuda and Saad, 2001). Weltzein (2000) explains how learning about farmers' preferences and selection criteria reoriented an international pearl millet breeding programme to identify components for the mixtures of plant types farmers customarily grow. The new materials were well-accepted by farmers who were not adapting modern varieties. A study conducted in Syria provides evidence of higher rates of adoption of PPB barley varieties. Farmers were planting 69 percent more area to PPB than to conventionally bred varieties and were willing to pay more for seed of PPB varieties (Lilja and Aw-Hassan, 2002). On average, farmers reported PPB varieties had a 26 percent yield advantage over conventionally bred varieties. In Ghana, maize breeders released several modern varieties, which had poor acceptance and were not widely adopted. Subsequently, new material was tested in researcher-managed trials and in farmer-managed trials, and the outstanding modern varieties were jointly selected. Overall adoption of modern varieties expanded to over two-thirds of Ghana's maize farmers (Morris, Tripp and Dankyi, 1999). Another study compared matched communities with and without PVS conducted by farmer research committees (CIALs). Communities doing PVS had a much higher rate of adoption than non-PVS communities, who relied on other channels for seed (IPRA, 2008). The WARDA PVS programme conducted in 17 West African countries since 1996 used consultative participation to understand better what farmers need, and to feed back insights to formal research for improving future on-farm productivity: 69 percent of national programme researchers considered that by consulting women and involving them in varietal evaluation, the

programme had included varietal traits that women know about, and especially gender-related varietal preferences, leading to higher adoption of the varieties.

Impact pathway: PPB leads to faster varietal release

A study that examines this impact pathway in depth was conducted by the ICARDA Barley Breeding programme in Syria (Ceccarelli *et al.*, 2001a). Using the same breeding population, varieties were developed using participatory and non-participatory breeding. The study found that by introducing farmer participation at an early stage of the breeding process (in Year 3), a three-year reduction was achieved in the time taken from initial crosses to release. PPB made certified varieties available by Year 6 compared to Year 9 in the conventional breeding programme (Lilja and Aw-Hassan, 2002)

PPB in rice and maize in India and Nepal found that farmers were well able to select from large numbers of segregating materials and their most preferred materials were rapidly adopted (Staphit, Joshi and Witcombe, 1996). Based on experience with different crops, the breeders concluded that PPB reduced by 3 to 4 years the time between making a cross and farmers receiving materials for testing. This contrasts with the conventional time of 10 years (Virk *et al.*, 2005, 2003)

In another case, farmer participation in screening the entire pearl millet germplasm accessions from Namibia (numbering about 1 000) proved very efficient in generating some basic information, such as when farmers recognized three major classes of materials with different clusters of desirable traits, and assisted breeders to come up with the desired pearl millet ideotype for Namibia. Breeders introduced material corresponding to the ideotype into farmer trials, and because millet is cross-pollinated, the frequency of the desired traits increased in local germplasm through introgression. Farmers began selecting outcrosses to provide seed for the following season, and after 4 years, breeders selected plants from a farmer's field. These plants were intercrossed with 30 varieties selected on-station by farmers from specially designed elite and morphologically diverse nurseries, to create a PPB composite population named MKC. MKC was far superior to the local germplasm and to another population, NC 90, developed by conventional breeding (Monyo *et al.*, 1997a, b).

PPB carried out in Bolivia addressed the need to develop potato varieties for specific ecological and market niches that need to be similar to those already valued by farmers and consumers, but more productive and more resistant to biotic factors such as Late blight disease (*Phytophthora infestans*) and False root-knot nematode (*Nacobbus aberrans*). Men and women farmers were trained in potato breeding techniques and, jointly with the plant breeders, generated 12 varieties similar to the most widely consumed cultivar in Bolivia, but resistant to late blight, with superior yield (10–25 t/ha, compared with 5 t/ha from the main farmer variety) and possessing agronomic traits and qualities desired by farmers. Three of the varieties showed novel potential for the potato chip industry. The breeders concluded that time was gained and adoption accelerated when farmers engaged early (Gabriel *et al.*, 2006).

Impact pathway: PPB's faster varietal release leading to earlier adoption increases the stream of benefits to farmers

An economic analysis of PPB barley breeding in Syria provides evidence of earlier

adoption impact. Over 23 PPB varieties are grown on several thousand hectares. Total estimated discounted research induced benefits to Syrian agriculture were estimated, comparing conventional and three different PPB approaches, based on a rigorous comparison using experimentally-generated data on yields. Benefits from conventional breeding were estimated at US$ 21.9 million. Benefits estimated for the three PPB approaches ranged from US$ 42.7 million to US$ 113.9 million. Most difference is attributed to the way PPB reduced the amount of time it took for improved varieties to get into farmers fields (Lilja and Aw-Hassan, 2002).

Impact pathway: more desirable varieties and higher adoption rates improve research efficiency

New Rice for Africa (NERICA) implemented by WARDA, the African Rice Centre, used PVS to evaluate new varieties with men and women farmers, and helped to identify cost-saving production, grain processing and consumption traits, in addition to yield-related characteristics, valued by men and women. Results from Côte d'Ivoire show that failing to include gender-differentiated production and consumption traits and focusing on the wrong attributes leads to biased and inappropriate varietal promotions. Evaluating new varieties only on yield-related characteristics (often gender-neutral) will cause 19 percent of all varieties to be wrongly categorized as superior, whereas incorporating gender-differentiated traits (labour-related, consumption, post-harvest) reduces the probability of promoting varieties with poor acceptability and instead increases adoption potential (Dalton and Guei, 2003; Dalton, 2004; Lilja and Erenstein, 2002; Lilja and Dalton, 1998).

One of the main concerns related to research efficiency of conventional breeding programmes is that PPB looks very time intensive, and therefore costly. Many aspects of PPB seem likely to increase costs: on-farm testing begins earlier, more seed is needed of experimental varieties, trials are dispersed outside the experiment stations, and different kinds of personnel may be needed to interact effectively with farmers. Farmers need to be transported to experiment stations or regional trials, and a good deal of time is spent interacting with them to involve them in the early stages of the breeding process. In the case of a high altitude rice in Nepal, Staphit and Subedi (1996) considered their combined PVS and PPB approach cost-effective because the parents and segregating products were 'piggybacked' off the ongoing formal breeding process. Farmers were given still segregating (F_5) bulk families harvested from the most promising F_4 rows, for evaluation in their fields. There were important differences in the ways farmers and breeders tested the materials. The preferred cultivars subsequently developed with farmers were widely adopted within three years. In ICARDA's study that compared PPB and conventional approaches the operational costs of the programme increased due to PPB, which included costs of work off station in Syria and in several other countries. However, operational costs are only 23 percent of the total budget. Overall, the total annual budget went up by 3 percent, approximately US$ 26 000. (Lilja and Aw-Hassan, 2002). This cost has to be seen against the savings incurred by getting varieties out to farmers three years earlier using PPB. Clearly more analyses of the way PPB affects costs would help to clarify this debate, but at present we cannot conclude that PPB automatically represents a major increase in cost for a breeding programme.

Impact pathway: PPB fosters new skills, new knowledge and social capital that speed up innovation

PPB involves moving off conveniently-located, well-endowed experiment stations to a more decentralized breeding programme that relies heavily on farmer-managed selection. Numerous studies conclude that selection by farmers offers the greatest yield benefit over experiment station selection in low-yield-potential, marginal environments that differ dramatically from experiment station conditions (Weltzein *et al*., 2001; Smith, Castillo and Gómez, 2001; Cecarelli, 2000, 2001, 2003; TAC, 2001; Virk *et al*., 2003; Morris and Bellon, 2004; Walker, 2006). Decentralization places heavy demands on breeders' time and other resources, unless a significant degree of delegation to farmers takes place. In this situation, farmers need to develop the skills and knowledge required to maintain research quality with minimal supervision. Low rates of varietal turnover or replacement have been proposed as an indicator that farmers are not able to access varieties appropriate to their needs and constraints, signalling that opportunities exist to start PPB (Walker, 2006; Brennan and Byerlee, 1991). Breeders also acquire new skills and knowledge through PPB that improve their capacity to sustain the varietal change needed to increase productivity. However, whether PPB fosters capacity to sustain this innovation remains a research gap in assessment of the impacts of PPB. Whether PPB will gain enough traction to achieve institutionalization in breeding programmes is still an open question: without institutionalization, PPB's wider impact on innovation systems may remain hypothetical. Social analysis of innovation processes involving PPB are therefore an opportunity for further research that could contribute to its wider recognition.

Although not assessments of PPB impact, several studies find that use of participatory research and learning approaches improve skills and knowledge important for innovation (see, for example, Dalton *et al.,* 2005; Classen *et al.,* 2008). For example, social and human capital benefits have been studied for members of farmer research committees (CIALs) in Latin America (Classen *et al.,* 2008). CIAL members indicated that they had gained more knowledge about agriculture and were seen as agricultural experts and advisors in the community. They had also improved their communication and leadership skills, and had increased relationships with neighbours and with other outside institutions. CIAL members experimented more with new crops, had learned other new skills, and had higher levels of commitment to their communities, thereby leading to a higher level of community participation. In communities where the CIAL had identified new technology and converted into commercial seed producers, the communities benefited by having easy access to new technology (e.g. new varieties, such as early maturing maize varieties and new bean varieties). One study specifically examines the argument that improvements in social capital from using a participatory approach reduces transaction costs, leading to better cooperation and coordination and improving the innovation process (Gandarillas Morales, 2007). Thus impact pathway remains poorly documented for PPB, reflecting the focus of most studies on the breeding products and outcomes rather than the social impacts of PPB. This therefore remains an area ripe for further social analysis.

Impact pathway: PPB increases inclusion of the poor and disadvantaged, especially women, in R&D, leading to more equitable distribution of benefits

This impact pathway remains a significant research gap in the assessment of PPB. Only if programmes make a specific strategy of including the poor or other disadvantaged groups in the process, will PPB or PVS lead to more equitable distribution of benefits. It is often wrongly assumed that use of participatory approaches will guarantee inclusion of the poor or women and lead to more equity. While these approaches make it easier to engage with the poor, unless participant selection targets a particular social group, a participatory approach does not automatically lead to benefits for them (Kumar and Corbridge, 2002; Cleaver, 1999). The implications for PPB are illustrated by the findings of a study assessing impacts of participation in potato Integrated Pest Management (IPM) and PPB in Peru (Ortiz *et al*., 2001). Women did not participate in potato IPM because pest management is not part of their traditional responsibilities in the potato crop, and they said they could participate more in other traditional women's crops. However, participatory selection of potato clones was identified as an activity in which women had an essential contribution, because they are responsible for seed management, and so they were 50 percent of the participants in varietal selection. In contrast, a study carried out in Ecuador with two indigenous communities, to determine farmers' preferences for quinoa cultivars and to improve PPB processes, found more women than men participated because quinoa, a primarily subsistence crop, is mainly managed by women (McElhinny *et al.,* 2007). An analysis of economic costs and benefits of participation by farmers in conserving and improving maize landraces in Mexico concluded that farmers as a group earned a high return with a benefit:cost ratio of 3.8-1 although for the private investor the returns were low. This underscores the importance working with groups of farmers to build participation. Participants from richer households captured a larger proportion than they invested so that there was a transfer of wealth to the richer households from the intermediate investors, also the largest sub-group of investors. This reinforced the gender bias in the distribution of benefits (Smale et al., 2003) The lesson here is that participation can lead to the exclusion of important groups of beneficiaries, such as women, depending on prevailing customs and norms, especially if participation is based on self-selection. Inclusion in PPB may be determined *a priori* by the choice of crop, suggesting that if equity is an impact goal, then the decision 'with whom' to do PPB should precede ones about where and which crop to work with.

25.4 CONCLUSIONS

PPB produces more acceptable varieties, increasing adoption. This is its most extensively documented outcome and probably the most compelling incentive for plant breeders to use this approach. While major PPB initiatives, such as ICARDA's, do document yield improvements of 30–50 percent with PPB, there remains a need for more comparative data on yield or other advantages of PPB versus other breeding approaches, data that could be used to assess final impacts. Another consideration is research efficiency: PPB makes research more relevant and the changes in cost it involves do not appear to lower breeding programme cost–benefit ratios, and might even improve these. PPB also affects the speed at which new varieties are developed

and get into farmers' fields. The important determinants of this impact are the use and timing of collaborative participation: early involvement of farmers in setting breeding goals encourages the co-production of new knowledge, gives farmers confidence in and ownership of the new varieties, and stimulates their rapid dissemination. To realize its full potential on a large scale, PPB requires organizational, policy and legal changes in both international and national plant breeding which, with few exceptions, represent tenacious obstacles to the institutionalization of PPB because science bureaucracies and the political elites that fund them, resist being accountable to poor farmers as clients. In the long term, one of the most important impacts of PPB may be its effect on the relevance of these agricultural innovation systems to the poor.

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This book provides a comprehensive description and assessment of the use of participatory plant breeding in developing countries. It is aimed at plant breeders, social scientists, students and practitioners interested in learning more about its use with the hope that they all will find a common ground to discuss ways in which plant breeding can be beneficial to all and can contribute to alleviate poverty.

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