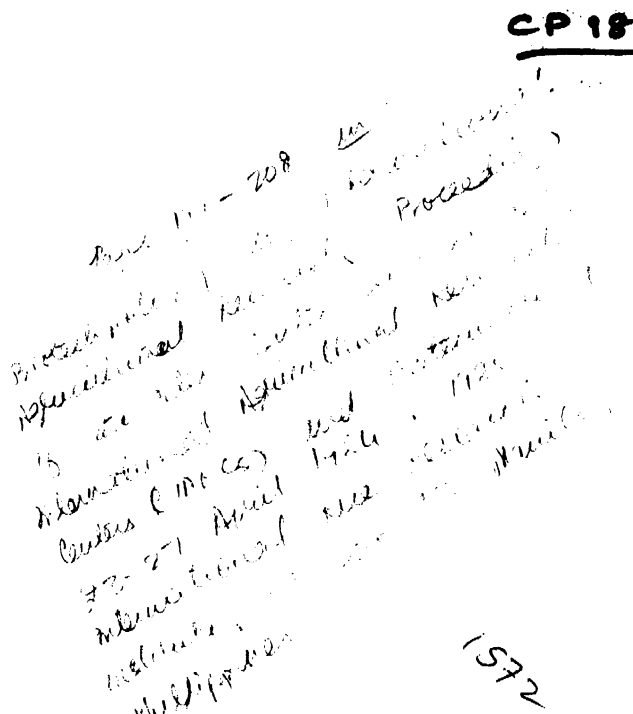

CP 185

WILD SPECIES IN CROP IMPROVEMENT

J. P. MOSS

Wild relatives are useful sources of characters desired to improve crop. Many of these species are resistant to pests and diseases or adapted to adverse environments. Some have higher yields than their cultivated relatives. Genes from some wild relatives can undergo meiotic recombination, producing segregants from which the plant breeder can select improved types; these genes can usually function effectively in cultivars. The limit to utilization of wild relatives depends on the breeder's ability to produce hybrids, but hybrids may be sterile. Ploidy differences between crops and wild relatives are frequent, but manipulating ploidy level can improve gene transfer. Triploids are often sterile, but progeny from triploids include recombinants that may not occur at other ploidy levels. The Groundnut Cytogenetics Unit at ICRISAT has transferred desirable genes from diploid wild species into tetraploid lines crossable with groundnut, enabling breeders to incorporate wild species genes into locally adapted material. Triploids, hexaploids, amphiploids and autotetraploids have been produced and backcrossed with the cultivated groundnut. Some lines have disease resistance and good agronomic traits, including high seed yield, and have been entered in All India Coordinated Oilseeds Project trials.

The aim of the plant breeder is to change one or more genes in a cultivar to better adapt it to its environment or to its intended use. These genes come from a range of sources. Most plant breeders use other cultivated germplasm. Few attempt to transfer genes from more distantly related germplasm even though wild relatives have many desirable attributes. Wild species have often survived pests and diseases, and have many resistance genes. They may grow in a wider range of environments, including harsh ones. There is now evidence that they can also contribute to yield increases.

All this can also be claimed for more exotic germplasm, but wild relatives have advantages over unrelated material. There may

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be genomes or parts of genomes common to cultivated and wild species, facilitating gene transfer. Wild genes may function in the genetic background of the cultivar; exotic genes may not.

There have been a number of recent reviews and books on wild species utilization (5, 27, 32), but relatively few people currently transfer genes from wild species.

This conference looks at new technology to make genes available to the breeder. This paper focuses on transferring genes of wild relatives to crops, emphasizing the work at ICRISAT using wild Arachis species to improve groundnut.

LIMITS TO THE GENE POOL

The difference between cultivated and wild is arbitrary. Many wild species are freely crossable with cultivars; indeed, some wild species scarcely differ from cultivated, but survive in the wild because of a single gene difference such as rachis fragility. One wild Arachis species, A. monticola, freely crossable with A. hypogaea, has been used to develop the cultivar Spancross (4). The next section of the gene pool is not readily accessible. Its limits generally follow taxonomic divisions; it may be restricted to one species, or span even several genera. The gene pool for wheat has increased greatly in recent years, and the breeder has a wide range of potential gene donors. Isolation may be due to crossability barriers and the inability to produce hybrids, or to hybrid sterility, preventing further gene flow. When fertile hybrids have been produced, lack of recombination may mean that undesirable characters are always expressed in the desired selections (28). When the gene has been transferred, gene interaction or pleiotropy may prevent selection of the desired new phenotypes. Alternatively, transferred genes may produce effects not present in the wild species (15). Only one of these barriers may be effective, but in some cases many may occur, and the more distantly related the species, the more problems are likely. Where a gene has been introduced and has undergone recombination within the cultivated genome, it can then recombine and be used by any breeder. A gene introduced by other means may not be amenable to traditional plant breeding.

This section of the gene pool comprises many species. Table 1 shows crop diversity in ploidy levels and numbers of wild relatives. Some wild species are represented by few accessions; others have been extensively collected.

SELECTION OF PARENTS

Having decided on the gene pool, assemble all knowledge of those species. Usually desirable genes will have first priority (31); if possible, the knowledge should extend to the nature and number of the genes in each species, and whether the gene is the same in all species. This knowledge can come from genetic studies, but they take time and assume easy crossability and full hybrid fertility; genetic ratios from interspecific hybrids will not be valid if meiosis is not normal. Another approach is to study mechanisms and components of resistance (30). Even where

Table 1. Major crops of the IARCs and their wild relatives.

Cultivated species	2n =	Ploidy level	Wild relatives
A. Sexually reproduced			
Phaseolus sp.	22	2x	Many; diploids - many cultivated species, and wild forms of cultivated species
Vigna unguiculata	22	2x	Many; diploids
Cajanus cajan	22	2x	Few; diploids
Vicia sp.	12	2x	Very many; different basic chromosome numbers, no interspecific hybrids involving *V. faba*
Glycine max	40	4x	Few; diploids, tetraploids
Cicer arietinum	16	2x	Few; diploids
Oryza sativa	14	2x	Many; mostly diploids, some tetraploids
T. aestivum	42	6x	Many wild relatives; diploids, tetraploids
T. durum	28	4x	and hexaploids. Widely crossable. Elymus, Sitanion, Haynaldia, Aegilops, Agropyron, Secale, Hordeum now part of gene pool
Zea mays	20	2x	Related genera, with different basic numbers and different ploidy levels
Sorghum vulgare	20	4x	Many; diploids, tetraploids
Pennisetum americanum	14	2x	Few; x=7 diploids, tetraploids
			Many; x=9 diploids, tetraploids
Arachis hypogaea	40	4x	Many; diploids, Few; tetraploids
B. Vegetatively reproduced: may be highly polyploid, triploid, aneuploid, also sterile and produce few seeds which germinate poorly.			
Solanum tuberosum	48	4x	Very many, at different ploidy levels
Ipomoea batatas	90	6x	Many, 2x, 4x
Xanthosoma spp.	26	2x	Few; (tetraploids occur)
Colocasia esculenta	28	2x	–
Manihot esculenta	36	4x	Few; tetraploids. Interspecific hybrids have been produced

resistance occurs in the cultivated species, resistance in the wild species may be controlled by different genes (23) that can be combined to give a more stable resistance. There is a very wide choice of cultivated germplasm (13); cultivars to be used as parents should be selected for crossability with the wild species as well as for local adaptation.

THE WILD SPECIES

A wild species consists of a number of plants in their natural habitat, but for the plant breeder it is the gene pool at his disposal: the number of accessions he can collect from the wild or acquire from gene banks. These accessions may differ from one another. The breeder should screen all of them for the desired character, bearing in mind that resistant selections may differ in genotype, or in resistance components (30). Crossability may differ, as it often does in the cultivated species (8, 9, 17, 24). A study of allozymes indicates the degree of variability in species (16), and karyotype studies will indicate similarities and differences between genomes (20). The variability may lead to

differences in chromosome behavior and hybrid fertility, which can result in changes from homologous to homoeologous pairing (2, 3) or differences in chiasma distribution.

About 1,000 collections from the wild have been made in Arachis, and a collection program supported by the International Board for Plant Genetic Resources is still active. Although not all collections have been maintained, ICRISAT has 181 accessions of wild Arachis species (14), and there is an active program to assemble all living accessions at ICRISAT. All the accessions are being screened for resistance to major pests, diseases, and drought (1, 29, 31).

Effective screening techniques are essential to wild species utilization, not only to screen accessions of wild species, but also to identify segregants with the desired characters. The infector row technique has been valuable in screening for resistance to rust (Puccinia arachidis) and late leafspot (Cercosporidium personatum).

The genus Arachis

There are seven sections in the genus. Arachis hypogaea, the cultivated groundnut, is in section Arachis. It is a tetraploid; A. monticola is also a tetraploid freely crossable with, and considered by some a subspecies of, A. hypogaea. All the other species in section Arachis are diploid, and crossable with A. hypogaea, but A. hypogaea will not cross with species in any other section, unless hormone treatment and embryo culture are used (18, 19). Diploid species in section Arachis can be crossed with species in other sections, but gene transfer by bridge crossing has not been possible (28).

ICRISAT has therefore emphasized work to overcome barriers to intersectional hybridization (18) and to utilize diploid wild species in section Arachis, especially those with disease resistance, by producing large populations of hybrids and backcrosses.

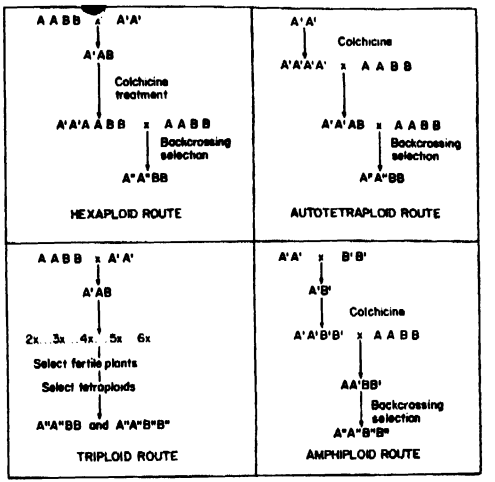
A knowledge of crossability and genomic constitution of the species is necessary in planning its utilization; most of this knowledge comes from hybrid production and analysis but knowledge of genomes can come from karyotype studies, DNA studies, and taxonomic studies.

Barriers to hybridization

The available gene pool is limited by the capability and resources of the breeder. Many species can be crossed by conventional means, but the use of mentor pollen, hormone treatment, and embryo culture can extend the pool (19).

Ploidy differences

Many crop species are polyploid, with diploid or polyploid wild relatives, or both. Existing ploidy differences impede gene transfer, as hybrids are often sterile, but induced ploidy can be used to overcome crossability barriers and to manipulate genome ratios to alter gene doses, and to effect gene transfer (22).



1. Use of related diploid species in improvement in *Arachis*. A, B = cultivated genomes, A', B' = wild genomes, A", B" = introgressed genomes.

Ploidy level differences between species can be adjusted before or after crossing (Fig. 1). A diploid wild species can be rendered tetraploid before crossing with the tetraploid crop (autotetraploid route) (6), or the tetraploid crop can be rendered dihaploid before crossing at the diploid level. Two diploid relatives, where available, can be crossed and the hybrid can be rendered tetraploid before crossing with the cultivated tetraploid (amphiploid route). The second diploid species may contribute another resistance (leafspot resistant A. cardenasii/rust resistant A. batizocoi)/A. hypogaea, or confer cytological stability and fertility on the tetraploid hybrids, thereby facilitating transfer of the desired gene. In Arachis, we have concentrated on eight diploid species, most of them with A. hypogaea as female parent; of the 72 possible species combinations we have produced 56; we have also crossed diploid hybrids with a third wild species and produced complex hybrids.

Often direct hybrids without ploidy manipulation are sterile. Many triploid hybrids produced in Arachis are totally or partially sterile (11, 12). Colchicine treatment produces fertile hexaploids (26). Wild and cultivated genomes are combined, but after treatment each genome is homologous, and there may be no pairing between wild and cultivated chromosomes. If the genomes are related, intergenomic pairing may occur, resulting in multivalents (25). Backcrossing alters genome ratios, removes

homologous partners, and encourages wild and cultivated chromosome pairing; but the recombinants may be released slowly, needing many generations for backcrossing and screening. After an initial backcross, chromosome numbers may continue to decline in selfed progenies, but there may be strong selection for balanced gametes, and pentaploids may give mostly tetraploids and hexaploids on selfing. Recombination may have occurred at the pentaploid level; pentaploid-derived tetraploids and hexaploids would then contain chromosomes with both wild and cultivated segments.

Though triploids are usually sterile, some seeds can be produced from anaphase distribution, giving balanced, viable gametes, or from restitution to produce triploid gametes. Chromosome numbers in progeny from such partially fertile triploids range from diploid to hexaploid (7, 21); tetraploid or near-tetraploid progeny are doubly valuable as restoring chromosome numbers to cultivated level requires little time and no colchicine or backcrossing, and recombination between wild and cultivated chromosomes, which may not occur at other ploidy levels (22), has been possible.

Recombination

The key to successful utilization of wild species is recombination; induced transfer of chromosome segments can be used, but producing a large number of hybrids in which meiotic recombination leads to large segregating populations gives more opportunity for the selection of desirable plants. Usually maximum recombination occurs in the first-generation hybrids. The difficulty in producing hybrids often results in small populations; a common report is that "a hybrid was produced; of the few F_2 plants obtained, only one was fertile." Vegetative reproduction of F_1 plants is essential in such cases to ensure acceptable population sizes. Tissue and cell culture at this stage may be valuable to introduce somaclonal variation (10). Where ploidy manipulation and the elimination of wild species characters are planned, changes in chromosome number or the occurrence of deletions, duplications, or translocations that affect chromosome pairing, chiasma frequency, or position (and hence the number and nature of recombinants in future generations) may be more important than the genetic changes that occur.

Backcrossing

It is hoped that the initial hybrids contain the desired gene. Usually because they combine both wild and cultivated genomes, they contain a number of undesirable wild characters also. Such characters can be eliminated by backcrossing to the cultivated species, although backcrossing to the wild species may be desirable. Hexaploids can be backcrossed to parental diploid wild species to regain tetraploidy and also increase the dosage of wild genes (4).

Backcrossing to the cultivated species restores cultivated chromosome number, where necessary, and also restores the genotype of the cultivated parent. Backcrossing to a nonparental cultivar can introduce alien genes; it will also give rise to variation in the background genotype in which the introduced gene is to express itself. This has been used extensively in the Groundnut Cytogenetics crossing program at ICRISAT. Long-season hexaploids with genomes from perennial Cercosporidium-resistant wild species and from Nigerian cultivars were backcrossed to short-season Indian cultivars; and resistant short-season selections were backcrossed to a Groundnut Rosette Virus-resistant cultivar from Malawi.

Cytological screening

The need for large populations precludes cytological screening of all plants. We currently grow about 7 ha of wild species derivatives each rainy season at ICRISAT, and have grown 12 ha in one season. Chromosome counting and checking for regular meiosis is restricted to key hybrids and any plants with unexpected morphology or reduced fertility.

Cytological stability

Ploidy differences and intergenomic pairing are useful in the early generations, but not in the finished product. Many generations of backcrossing may be necessary to select stable lines with the desired character and no deleterious wild species genes. Selected plants can be selfed, and progeny rows grown for several generations until uniform lines are produced. Chromosome numbers are counted and meiosis is checked at this stage. Lines for release as new germplasm must be checked for crossability with the cultivars and for regular meiosis in the hybrids produced.

Table 2. Disease reaction (1-9 scale) and yield of selected wild species derivatives in *Arachis* (8 x 8 triple lattice, plot size 16 m^2), ICRISAT Center and Bhavanisagar, 1982 rainy season.

Entry no.	Rust resistance	Leaf spot[a] resistance	Pod yield (kg/ha)	Estimated kernel yield (kg/ha)	Oil content (%)	Estimated oil yield (kg/ha)	Haulm yield (kg/ha)
13	2.3	2.3	3150	1960	42	820	4570
30	2.7	2.3	2640	1700	44	760	6540
46	6.7	2.3	2540	1740	44	770	4490
6	2.7	2.3	2150	1360	43	580	3700
11	2.7	2.0	2520	1750	44	770	5620
23	3.0	3.0	2140	1410	44	620	5080
39	9.0	2.3	2130	1370	45	610	3270
SE	±0.42	±0.55	±134	±87	±0.7	±39	±350
Check							
Robut 33-1	9.0	6.3	1830	1320	41	510	1580
TMV2	9.0	9.0	1240	850	41	350	1630
SE	±0.0	±0.24	±80	±60	±0.3	±24	±53
Site mean[b]	4.2	5.5	1950	1280	43	550	4050
CV (%)	17	17	12	12	3	12	15

[a]Results from trial at Bhavanisagar. [b]Mean of 64 entries (ICRISAT Center) 36 entries (Bhavanisagar).

ACHIEVEMENTS

Many fertile, cytologically stable derivatives with desirable characters from wild species have been selected (7). The numbers of segregants produced from crosses have permitted selection for agronomic traits (growth, habit, yield, earliness) as well as for disease resistance. The most advanced lines have been grown in replicated trials for a number of seasons at ICRISAT center and other locations (Table 2). The best lines have been entered in All India Coordinated Oilseeds Research Project trials. Two foliar disease-resistant lines have been selected for a second year of testing, and one high-yielding line has been advanced to regional trials.

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OVERCOMING INCOMPATIBILITY IN WIDE CROSSES

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Wild species of crop plants attract much attention as a valuable gene pool. A few successes in improving crop plants have resulted from crossing wild taxa with cultivated ones. Several other taxa are not crossable with their cultivated relatives and are therefore unavailable for sexual gene transfers. Methods for breaking these barriers to interspecific hybridization and hybrid production have been developed.

The literature on exotic germplasm abounds with examples genetic introgression from wild taxa conventionally crossed wi their cultivated relatives. But there is a wealth of germpla that cannot be crossed with cultivated taxa.

The barriers to hybridization were known even before t present range of germplasm became available. During the la five decades, interest in incompatibility has increased encompass phylogenetic-taxonomic purposes and the genet improvement of crop plants. While emerging somatic methods sh promise, sexual manipulation is still the first choice, a sexual methods have contributed significantly to improving crop

The germplasm amenable to sexual manipulation is difficu to quantify. It may be an insignificant proportion of existi germplasm. Even in the well-worked genus Nicotiana, of whi about 65 species are known, only a little more than 300 hybri have been realized. About 90% of the crosses in this genus ha not. The situation is similar for most other crops. A number review articles and books on incompatibility and methods to bre it are available (9, 15, 16, 17, 18, 41, 49, 51).

CAUSES OF SEED FAILURE IN INCOMPATIBLE CROSSES

The characteristic prefertilization barriers postfertilization breakdown of zygote or embryo in incompati crosses have been extensively described. Inhibition of pol germination on stigma and pollen tube growth through the sty

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