

3 Peanut

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3.1 Introduction

3.1.1 Origin

The legume genus *Arachis* is of South American origin and contains about 80 known species with natural distributions restricted to Brazil, Bolivia, Paraguay, Argentina, and Uruguay (Valls and Simpson 1994).

These wild *Arachis* species are divided into nine taxonomical sections, based upon morphology and sexual compatibilities. *A. hypogaea*, the cultivated tetraploid peanut (also known as groundnut), is found in the section *Arachis*, along with some 25 wild diploid species. This section also contains another tetraploid species, *A. monticola*, which readily hybridizes with *A. hypogaea*, is almost indistinguishable using DNA markers, and may best be considered as conspecific.

It seems that the origin of *A. hypogaea* was through the hybridization of two diploid species with distinct genomes giving rise to a sterile hybrid. A spontaneous duplication of chromosomes restored fertility, but left the plant reproductively isolated from its wild relatives (Kochert et al. 1991; Jung et al. 2003; Seijo et al. 2004). It is most likely that these events occurred once or only a few times. There is doubt about exactly which diploid species were involved and as to where these events occurred. However, it seems logical that the diploid species involved were probably brought together by human action through the cultivation of species that have distinct and separate natural distributions. In support of this, archaeological finds of shells of fruits closely resembling *A. duranensis* Krapov. and W.C. Gregory; *A. magna* Krapov., W.C. Gregory and C.E.

Simpson; *A. ipaensis* Krapov. and W.C. Gregory; and *A. monticola* Krapov. and Rigoni were excavated near Casma in coastal Peru. These species today are considered entirely wild but were apparently cultivated in the remote past. It is not necessarily the case that the origin of *A. hypogaea* was in this region; indeed, it seems that this would be more likely to have happened in the Eastern slopes of the Andes where *A. monticola* exists today in a wild state. The climate in this region is moister, not as good for the preservation of archaeological remains, but more favorable for plant growth, and a better environment for the wild bees that would have done the necessary initial hybridization (Simpson et al. 2001).

3.1.2 Botanical Types and Distribution

A. hypogaea is divided into two subspecies, *hypogaea* and *fastigiata*, and six botanical varieties (Krapovickas and Gregory 1994). The subsp. *hypogaea* var. *hypogaea* has a long cycle, no flowers on the central stem, and regularly alternating vegetative and reproductive side branches. It is exemplified by the Virginia types that are widely present along the tributaries of the right margin of the Amazon Basin in Brazil and Bolivia. Also classified within subsp. *hypogaea*, but with more hirsute leaflets and even longer cycle, is the variety *hirsuta* Kohlér. Nowadays this variety is concentrated in the coastal regions of Peru, from where it extends to Central America and Mexico, Asia, and Madagascar. The variability of this variety found in the Old World even suggests the possibility of pre-Colombian contacts (Simpson et al. 2001).

The subspecies *fastigiata* Waldron has a shorter cycle, flowers on the central stem, and reproductive and vegetative shoots distributed in a disorganized way. This subspecies includes four varieties. The variety *vulgaris* C. Harz has its distribution/spread along the basin of the river Uruguay, usually the fruits are two seeded, and correspond to the agricultural type Spanish. The variety *fastigiata* has fruits with more than two seeds and a smooth pericarp. This variety corresponds to the agricultural type Valencia; centers of diversity are in Paraguay and in central and northeastern Brazil extending to Peru. The other two varieties, *aequatoriana* Krapov. and W. C. Gregory (Equador and North of Peru) and *peruviana* Krapov. and W.C. Gregory (Peru, northeast Bolivia, and the Brazilian state of Acre), have fruits with more than two seeds, heavy reticulation of the pericarp, and very restricted distributions (Krapovickas and Gregory 1994).

In addition, Brazilian germplasm includes material that is difficult to fit into the above classification. Particularly notable in this respect is material from the Xingu river basin (Freitas and Valls 2001).

Modern cultivars generally have a narrow genetic base (Isleib et al. 2001). Nevertheless they are generally classified into the agricultural types Spanish, Valencia, or Virginia. These cultivars have been widely used as representatives of the botanical varieties in analyses of genetic variability. These cultivars often have their origin in more than one variety or subspecies and are not correct taxonomic representatives; their use as such may lead to incorrect conclusions.

3.1.3

Crop Production and Uses

Peanut is the major oilseed crop in the world, grown on 26 million ha producing nearly 36 million tons annually. Although the global average productivity is low (1.35 t ha^{-1}), many countries achieve much higher levels of productivity, including the USA (3.54 t ha^{-1}) and China (2.62 t ha^{-1}) (FAO 2003). Developing countries contribute about 94% of the world peanut production, grown mostly under rainfed conditions (predominantly in Asia and Africa) (Table 1). Analysis of peanut productivity from the 1960s to the 1990s reveals interesting profiles across regions and in specific countries. While the average global

peanut productivity has steadily increased during that period, from 8% in the 1970s (average yield 0.85 t ha^{-1} in the 1960s) to 18% each in the 1980s (average yield 0.92 t ha^{-1} in the 1970s) and 1990s (average yield 1.09 t ha^{-1} in the 1980s), changes in productivity have been highly variable across different regions. For example, peanut yield in Asia increased by 14% in the 1970s (average yield 0.82 t ha^{-1} in the 1960s), 20% in the 1980s (average yield 0.93 t ha^{-1} in the 1970s), and 32% in the 1990s (average yield 1.12 t ha^{-1} in the 1980s), while in Africa productivity has largely stagnated or even declined during certain periods. In north Central America, tremendous yield increases (45%) were achieved during the 1970s (average yield 1.62 t ha^{-1} in the 1960s) but thereafter only marginal increases were seen as the importance of the crop in this region began to decline. A similar trend was seen in the USA with 54% increase in peanut productivity during the 1970s (average yield 1.73 t ha^{-1} in the 1960s) but little increase thereafter due to emphasis on stabilizing the yield by incorporating resistance/tolerance to pests and diseases and improving seed quality. In contrast, peanut yield remained stagnated during the 1960s and 1970s in South America but then increased by 28% in the 1980s (average yield 1.24 t ha^{-1} in the 1970s) and 16% in 1990s (average yield 1.58 t ha^{-1} in the 1980s). The peanut yield in Argentina and Brazil remained stagnated in the 1960s and 1970s but registered a 89% increase in Argentina (average yield 1.16 t ha^{-1} in the 1970s) and a 31% increase in Brazil (average yield 1.32 t ha^{-1} in the 1970s) in the 1980s and remained stagnated at this level in the 1990s. The greatest sustained improvement has been seen in China with a 19% increase during the 1970s (average yield 1.03 t ha^{-1} in the 1960s), 47% in the 1980s (average yield 1.23 t ha^{-1} in the 1970s), and 41% in the 1990s (average yield 1.81 t ha^{-1} in the 1980s). These statistics seem to indicate a direct correlation of perceived national economic importance (and presumably in turn investment in research and breeding) with increases in productivity. The prevalence of biotic and abiotic stresses and the level of technological innovation at the farm level are probably a major source of yield variation observed across regions and between countries within regions. However, it is equally inevitable that a major portion can be attributed to progress in genetics and breeding research that have been captured in new varieties. This is surely a strong justification for increased investment in plant research for genetics

Table 1. Area, production and productivity of the peanut across globe, regions, and major peanut producing countries within the region (FAO 2003)

Region	Country within region	Area (000 ha)	Production (000 t)	Average yield (t ha ⁻¹)
Asia		14988.60 (56.64) ⁺	24000.55 (67.31) ⁺	1.60
	India	8000.00 (53.37) ⁺⁺	7500.00 (31.25) ⁺⁺	0.94
	China	5125.40 (34.19)	13447.45 (56.03)	2.62
	Indonesia	682.94 (4.56)	1377.00 (5.74)	2.02
	Myanmar	575.00 (3.84)	730.00 (3.04)	1.27
	Vietnam	240.30 (1.60)	400.00 (1.67)	1.66
Africa		10472.57 (39.57)	8969.19 (25.15)	0.86
	Nigeria	2800.00 (26.74)	2700.00 (30.10)	0.96
	Sudan	1900.00 (18.14)	1200.00 (13.38)	0.63
	Senegal	900.00 (8.59)	900.00 (10.03)	1.00
	Chad	480.00 (4.58)	450.00 (5.02)	0.94
	Congo (DR)	456.59 (4.36)	355.18 (3.96)	0.78
	Burkina Fasso	331.00 (3.16)	301.00 (3.55)	0.91
	Mozambique	292.54 (2.79)	109.92 (1.22)	0.38
	Cameroon	283.00 (2.70)	294.89 (3.29)	1.04
	Zimbabwe	260.00 (2.48)	125.00 (1.39)	0.48
	Niger	230.00 (2.19)	100.00 (1.11)	0.43
North Central America		657.10 (2.48)	2077.77 (5.83)	3.16
	USA	530.95 (80.80)	1879.75 (90.47)	3.54
	Mexico	62.01 (9.44)	74.64 (3.57)	1.20
South America		307.08 (1.16)	559.92 (1.57)	1.82
	Argentina	156.40 (50.93)	315.60 (56.36)	2.02
	Brazil	85.02 (27.68)	177.06 (31.62)	2.08
Oceania		25.76 (0.097)	40.88 (0.12)	1.59
	Australia	22.00 (85.40)	37.00 (90.51)	1.68
World		26462.86	35658.43	1.35

⁺ % of global area and production

⁺⁺ % of region area and production

and genomics-enhanced peanut-breeding programs. China, the USA, Argentina, and Vietnam together contribute 75.5% of the world shelled peanut trade (1.13 million tons), with a total export value of US\$ 593.6 million (FAO 2002). The US shelled peanut seeds command a premium price of US\$ 786 per metric ton in the international market.

Abiotic and biotic stresses are the major constraints on world peanut production and are extensively reviewed in Dwivedi et al. (2003a). In addition, devastating new diseases are also emerging. For example, a new disease diagnosed as peanut stem necrosis disease (PSND) caused by tobacco streak virus (TSV) affected nearly 225,000 hapeanut crops that resulted in yield losses of over US\$65 million in India during 2000 (Rao et al. 2003a).

Peanut is a rich source of oil, protein, minerals (Ca, Mg, P, and K), and vitamins (E, K, and B₁) (Savage and Keenan 1994). Freeman et al. (1999) predicted a continued increase in peanut production in Asia, a slow increase in sub-Saharan Africa, and decline in Latin America. There will be a gradual shift away from peanut oil and meals to peanut confectionary products in Asia, Latin America, and the Caribbean. The cake remaining after oil extraction is used in human food or incorporated into animal feeds (Savage and Keenan 1994). Peanut haulm is excellent forage for cattle as it is rich in protein and more palatable than many other fodders (Cook and Crosthwaite 1994). Wild *Arachis* species are used in pasture improvement in the Americas and Australia (Kerridge and Hardy 1994).

3.1.4

Improved Quality Requirements: Reduced Allergenicity and Toxicity

Peanut is widely used in the food and confectionery industry due to its high nutritive value. However, it is well known for its allergenic properties affecting both children and adults. A study in the USA revealed that about 3 million American children and adults suffer from allergy to peanut or tree nuts (Sicherer et al. 1999). Most importantly, just a trace of peanut can provoke an abnormal IgE-mediated immunological reaction ranging from nausea or drowsiness and vomiting to anaphylactic shock and death. Peanut has seven distinct allergenic proteins, Ara h1 to Ara h7 (Burks et al. 1991, 1992; De Jong et al. 1998; Kleber-Janke et al. 1999; Herman 2004), that together include the vast majority of proteins in seeds and include both storage protein families (Koppelman et al. 2001). Ara h1 and Ara h2 are major allergens (Burks et al. 1995; Stanley et al. 1997; Kleber-Janke et al. 1999) and the remaining five are minor allergens (Kleber-Janke et al. 1999). North American populations are more prone to Ara h1 than the European populations (Koppelman et al. 2001). A vaccine that can protect people from peanut allergies has been developed, and tests revealed that the vaccine effectively protected mice from peanut allergies, which provides some hope that we should be able to protect humans from peanut allergy (<http://www.nature.com/reviews/immunol>).

Quality characters are also of great importance in peanut production. For example, *Aspergillus flavus* Link ex Fries infection does not significantly affect peanut yields but its production of aflatoxins makes contaminated grain dangerous for animal and human consumption. The presence of aflatoxins also dramatically influences the marketing of peanut kernels and cake because of stringent international standards for permissible levels of aflatoxin contamination set by importing countries in Europe, North America, and Australia.

3.2

Genetic Resources in Peanut

There are a number of review articles covering the genetic resources of cultivated and wild *Arachis* species (Singh and Simpson 1994; Stalker and Simpson 1995; Dwivedi et al. 2003a; Holbrook and Stalker 2003; Pa-

terson et al. 2004). Thus in this section we will only provide a brief overview of the current status of genetic resources being maintained in national and international gene banks and the range of wild and cultivated accessions with beneficial traits.

3.2.1

Wild *Arachis* Species and Interspecific Gene Introgression into Cultivated Peanut

ICRISAT gene bank maintains 453 accessions, representing 9 sections and 44 wild *Arachis* species. Of these, 352 accessions belonging to 41 species are seed producing, 100 accessions of 2 species are vegetatively propagated, and 1 accession (*A. batizogaea*) is a natural hybrid (seed producing). One hundred ninety-five accessions of 16 species are annual and 232 accessions of 17 species are perennial. Information on 26 accessions of 11 species is not known. The gene bank acquired 418 accessions as donations from 8 cooperators in 4 countries (Brazil 22, United Kingdom 3, India 8, USA 285) and 31 accessions through 4 collecting missions in Brazil (Rao et al. 2003b; <http://www.icrisat.org/GroundNut/Arachis/Start.htm>). The whereabouts of the remaining four accessions maintained in the ICRISAT gene bank are not known. The Southern Regional Plant Introduction Station, USDA-ARS at Griffin, GA, USA, maintains over 700 accessions of 60 wild *Arachis* species (USDA-ARS 2002; http://www.ars-grin.gov/cgi-bin/npgs/html/site_holding.pl?S9). Large collections of wild *Arachis* species are also maintained at Texas A&M and North Carolina State University, Raleigh, NC, USA. The National Center of Genetic Resources (CENARGEN) in Brazil maintains over 1200 accessions of 81 species belonging to 9 sections.

Unlike cultivated peanut germplasm, wild *Arachis* species are reported to possess high levels of resistance to rust, leaf spots, nematodes, peanut bud necrosis virus (PBNV), tomato spotted wilt virus (TSWV), groundnut rosette virus (GRV) and groundnut rosette assistor virus (GRAV), leaf miner, *Spodoptera*, aphids, thrips, and jassids (Stalker and Simpson 1995; Dwivedi et al. 2003a; Rao et al. 2003b). Wild *Arachis* species are also reported to show wide variation for most of the morphological traits (Singh et al. 1996; Chandran and Pandya 2000). ICRISAT scientists characterized/evaluated 267 wild *Arachis* accessions from 37 species under greenhouse

conditions for 33 qualitative and 15 quantitative traits. The frequency distribution of 17 qualitative descriptors was uniformly distributed whereas for the other 16 traits it was skewed (70% or more) toward one class. These species, except for the height of the main stem, stem thickness, and basal leaflet on the main stem, showed large variation for lateral branches, plant width, stipule length, adnation of stipule on the main stem, petiole length on the main stem, apical leaflet length and width on the main stem, apical length and width on the primary lateral, hypanthium length, standard petal length, and peg length as revealed by the Shanon-Weaver diversity index that ranged from 0.022 for hairiness on the margin of the stipule of the main stem to 0.836 for basal leaflet shape on the primary lateral (H.D. Upadhyaya, ICRISAT unpubl. data).

The primary gene pool in peanut consists of accessions that belong to cultivated peanut (*Arachis hypogaea*) and the wild tetraploid species *A. monticola*. The secondary gene pool is represented by diploid species of the section *Arachis* that are cross compatible with cultivated peanut, while the tertiary gene pool includes species of the other sections that cannot be hybridized with *A. hypogaea* by conventional means. Both pre- and postzygotic hybridization barriers have been shown to restrict crossing between *Arachis* species. Despite the crossing barriers, several interspecific tetraploid derivatives have been developed that possess high levels of resistance to rust, early leaf spot (ELS), late leaf spot (LLS), nematodes, southern corn rootworm, corn earworm, *Spodoptera*, and jassids (see Dwivedi et al. 2003a and references therein) that are semi-improved genetic resources that researchers may use for mapping and genetic enhancement in peanut. From the interspecific crossing, two root-knot nematode resistant peanut lines, Coan and Nema TAM, have been released for cultivation in areas heavily infested with nematodes in USA (Simpson and Starr 2001; Simpson et al. 2003).

3.2.2

Cultivated Germplasm

The world's largest peanut collection of 14,966 accessions from 93 countries is housed at the RS Paroda Gene Bank in ICRISAT, Patancheru, India. This collection represents six botanical varieties: 45.8% var. *hypogaea* (6,838 accessions), 36.6% var. *vulgaris* (5,493 accessions), 15.7% var.

fastigiata (2,351 accessions), 0.1% var. *aequitronia* (14 accessions), 0.13% var. *hirsuta* (19 accessions), and 1.7% var. *peruviana* (251 accessions). Approximately 43% of the collection consists of landrace germplasm, 7% cultivars, 31% breeding lines, and 19% other genetic stocks (mutants and experimental germplasm) (Upadhyaya et al. 2001a). Passport and characterization data are accessible through the internet (<http://www.icrisat.org/GroundNut/Project1/pfirst.asp?gname=entire>) and the germplasm is freely available for distribution providing the requisitioned signs a material transfer agreement with ICRISAT. Other gene banks holding sizable numbers of cultivated peanut accessions are 9,027 accessions at USDA Southern Plant Introduction Center, Griffin, GA, USA; about half of these collections are unimproved landraces collected in the crop's centers of diversity in South America (Holbrook 2001).

A series of descriptors has been developed for standardizing the characterization of peanut genetic resources using various morphophysiological, reproductive, and biochemical traits (IBPGR and ICRISAT 1992). The majority of the ICRISAT peanut germplasm showed a large variation for qualitative and quantitative traits, seed quality traits, and resistance to biotic and abiotic stresses (Upadhyaya et al. 2001a). Field evaluation of these germplasms identified a large number of accessions possessing tolerance to drought and resistance to biotic stresses (Table 2). Some of these genetic resources have been used in breeding programs to develop improved breeding lines/cultivars resulting in significant economic gains to peanut farmers. For example, the largest impacts have been from the development of cultivars with resistance to sclerotinia blight (*Sclerotinia minor* Jagger), the peanut root-knot nematode [*Meloidogyne arenaria* (Neal) Chitwood race 1], and TSWV, which had an estimated economic impact of more than US\$ 200 million annually for US peanut farmers (Holbrook 2001).

3.2.3

Core Collections

The development of a core collection could facilitate easier access to peanut genetic resources, improve the efficiency of germplasm evaluations by reducing the number of accessions to be evaluated while increasing the probability of locating genes of interest thus

Table 2. Sources of resistance to rust, leaf spots, sclerotinia blight, groundnut rosette virus, aflatoxin, nematode, defoliator, aphid, and drought reported in cultivated and wild *Arachis* species

Trait	Peanut accessions with beneficial traits reported			
	Cultivated species	Reference	Wild <i>Arachis</i> species	Reference
Rust	169	Singh et al. 1997	29	Subrahmanyam et al. 1995
Late leaf spot	69	Singh et al. 1997	27	Upadhyaya et al. 2001a
Early leaf spot	37	Singh et al. 1997	11	Upadhyaya et al. 2001a
Groundnut rosette virus	116	Subrahmanyam et al. 1998	12	Subrahmanyam et al. 2001
Nematode	21	Holbrook et al. 2000	–	–
Seed infection and/or aflatoxin production by <i>Aspergillus flavus</i>	21	Singh et al. 1997	4	Thakur et al. 2000
Sclerotinia blight	50	Damicone et al. 2003	–	–
Defoliator (Leaf miner and <i>Spodoptera</i>)	9	Dwivedi et al. 1993; Wightman and Rao 1994; Rao and Wightman 1999; Stalker and Lynch 2002	38 (leaf miner) and 67 (<i>Spodoptera</i>)	Wightman and Rao 1994; Lynch and Mack 1995
Aphid	EC 36892 and ICG 12991	Padagham et al. 1990; Minja et al. 1999	Wild species not evaluated	
Drought	40	Nigam et al. 2003b; Seetharama et al. 2003	Wild species not evaluated	

enhancing their use in crop improvement programs, and simplify the gene bank management.

A very small proportion of the germplasm accessions are being used in peanut-breeding programs (Upadhyaya et al. 2002a). At ICRISAT, where about 14,966 accessions of cultivated peanut and 453 accessions of wild *Arachis* are available for use, only 132 cultivated germplasm and 10 wild accessions have been used in developing 8,279 breeding lines in 17 years from 1986 to 2002 (H.D. Upadhyaya, ICRISAT, unpubl. data). Few accessions have been extensively used in breeding programs: Chico (ICG 476) 1180 times and Robut 33-1 also known as Kadiri 3 (ICG 799) 3,096 times.

Holbrook et al. (1993) were the first to develop a core collection of 831 accessions from a set of 7432 USA peanut germplasms based on 6 morphological variables. Subsequently, a global core consisting of 1,704 accessions (14 morphological descriptors on 14,310 accessions) (Upadhyaya et al. 2003) and an Asia region core of 504 accessions (15 morphological descriptors on 4,738 accessions) (Upadhyaya et al. 2001b) were developed. However, when the size of col-

lection is too large and a core collection (10% of entire collection) becomes unmanageable, Upadhyaya and Ortiz (2001) suggested a strategy to select a minicore collection (10% of core or 1% of entire collection). Using this strategy, Upadhyaya et al. (2002a) developed a minicore consisting of 184 accessions (based on 16 to 18 agronomic and quality traits scored on 1704 core collection accessions in 2 contrasting seasons) that captured variability present in the core collection (1,704 accessions) and also in the entire collection (14,310 accessions).

Holbrook and Dong (2003) evaluated the USDA peanut core collection (831 accessions) for 16 morphological traits to develop a core of the core consisting of 111 accessions demonstrating that the genetic variation expressed in the core had been preserved in this core of the core collection. The accessions included in the core potentially possess new sources of variation for economically important traits. When the peanut global core, Asia region core and/or minicore collections, were evaluated for various traits in multi-environment trials, ICRISAT scientists identified new sources

of early maturity (21 accessions) (Upadhyaya et al. 2006) and tolerance to low temperature (12°C) at germination (158 accessions) from the global core (Upadhyaya et al. 2001a), for drought-tolerance traits (18 accessions) from the minicore (Upadhyaya 2005), and 60 accessions (15 *fastigiata*, 20 *vulgaris*, and 25 *hypogaea*) from the Asia region core that showed high yield potential, greater meat content (also known as shelling percentage), and 100-seed weight (Upadhyaya et al. 2005). These new accessions have trait-specific characteristics similar to the best control, for example Chico, for early maturity but were agronomically similar or superior but diverse. The use of these diverse sources would help in bringing in much needed diversity and broaden the genetic base of cultivars (Upadhyaya 2005). Similarly, when the USDA peanut core collection was evaluated, 21 accessions were resistant to peanut root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood race 1 (Holbrook et al. 2000); 55 accessions were resistant to TSWV (Anderson et al. 1996); 11 and 12 accessions were resistant to cylindrocladium black rot [*Cylindrocladum crotalariae* (Loos) Bell and Sobers] and ELS, respectively (Isleib et al. 1995); 6 accessions exhibited a 90% reduction in preharvest aflatoxin contamination (Holbrook et al. 1998); 6 accessions were resistant to rhizoctonia limb rot (*Rhizoctonia solanii* Kuhn) (Franke et al. 1999); and 20 and 30 accessions were classified as highly resistant (no disease) and resistant (<10% disease incidence) to sclerotinia blight [*Sclerotinia minor* Jagger and *Sclerotinia sclerotiorum* (Lib) de Bary], whereas 10 accessions were resistant to pepper spot [*Leptosphaerulina crassiasca* (Sechet) Jackson and Bell] (Damicone et al. 2003). These new sources performed better than or similar to the best cultivars for the target traits but were known to be diverse from all previous sources of those traits. Thus, these accessions are a good resource for analyzing genetic relationships and detecting allelic variation associated with beneficial traits through association analysis. Moreover, where allelic tests indicate an independent genetic basis for a given trait, there is an opportunity for pyramiding genes to enhance the level of expression of that trait, provided the genetic basis is highly additive and epistatically positive.

It is noteworthy that the core and minicore collections discussed in this section do not include accessions from wild *Arachis* species. These genotypes tend to have a much longer cropping duration than the cultivated germplasm and need special care when

grown under field conditions outside their natural habitat. Thus, it is very difficult to conduct routine large-scale evaluation trials that include both wild and cultivated material. However, there are considerable ongoing efforts focused on the characterization of wild accessions for various morphophysiological, reproductive, and quality traits (Sect. 2.1) that should lead to increased exploitation of wild germplasm in peanut-breeding programs.

3.3 Appropriate Germplasm and Evaluation Systems for Mapping Economically Important Traits in Peanut

The efficiency of marker-assisted selection (MAS) is highly dependent on the quality of the prior mapping process, which requires parental genotypes with substantially contrasting phenotypes (for the target trait), highly accurate and precise evaluation techniques, and large mapping populations. In addition, the cost-effective application of MAS requires one marker very close to (or preferably within) the gene of interest and two markers closely flanking either side and that these markers be based on simple robust PCR-based marker assays. In this section, we provide an overview of genetically diverse sources of economically important traits that may be useful in genome mapping and marker-assisted genetic enhancement in peanut. Then we briefly discuss the mechanisms and genetics of resistance/tolerance to important biotic and abiotic stresses and the key steps involved in generating accurate phenotyping data for those target traits.

3.3.1 Phenotypic Screens, Resistance/Tolerance Mechanism, and Genetics

Rust and Leaf Spots

It is essential that a pure disease inoculum be used for inoculating the test materials. This should be generated and maintained on incubated, inoculated, detached leaves of a highly susceptible cultivar such as TMV 2 in a plant growth chamber using a temperature of 23 °C and 12-h photoperiod. The early leaf spot

(ELS) and late leaf spot (LLS) conidia and rust urediniospores should be harvested with a cyclone spore collector and used for inoculation of the test materials separately.

A two-step evaluation is suggested for phenotyping: the field and glasshouse evaluation. The former is useful as a first step wherein a large number of genotypes/mapping populations can be evaluated for overall disease score and related to yield loss, whereas the latter is recommended for dissecting the components of resistance under more controlled conditions.

For field evaluation, the infector row technique is adapted wherein the resistant and susceptible controls are sown alternately every five rows of test materials. Sunflower is sown in a 4-m wide strip around the experimental plot to avoid aerial contamination by other foliar fungal pathogens. At 35 d after sowing (DAS) the infector row and the test materials are inoculated with disease inoculum containing either 20,000 ml⁻¹ rust urediniospores or 20,000 ELS or LLS conidia, late in the evening. Prior to fungal inoculation, the whole experimental field is given light overhead sprinkler irrigation for 30 min to create leaf wetness on the foliage throughout the night. Thereafter, overhead irrigation is given every day for 30 min in the late evening for 10 d to maintain alternate wet (night) and dry (day) periods to obtain maximum disease development (Butler et al. 1994). The experimental plot should be protected against leaf spots when screening for resistance to rust [using weekly application of Chlorothalonil (Kavach) at 2 g l⁻¹ water (500 l ha⁻¹)] while rust should be controlled when screening for resistance to leaf spots [using weekly application of calixin at 0.5 ml l⁻¹ water (500 l ha⁻¹)]. There is no fungicide that gives differential protection (i.e., protection against ELS in LLS screening trial and protection against LLS in ELS screening trial), so the phenotyping should be conducted in hot spots prone only either to ELS or LLS. A nine-point modified scale is used, where 1 = no disease and 9 = 81 to 100% disease (Subrahmanyam et al. 1995), to record % leaf area damaged, % leaf defoliation (in case of leaf spots), and disease scored at 15-d intervals from 35 d after inoculation until 1 week before harvest.

For greenhouse evaluation, the test materials along with susceptible and resistant controls are grown in 15-cm-diameter plastic pots containing autoclaved soil and farmyard manure (v/v 4:1 ratio). The inoculum procedure is the same as described in the case of field screening. Immediately after

inoculation, the pots are shifted into dew chambers (Clifford 1973) at 23 °C to ensure wetness of the leaf surface during the night. The pots are removed from the dew chambers on the morning of the following day and returned to the greenhouse to maintain a dry period during the day. This alternation of wet (16 h) and dry (8 h) periods is repeated for 10 d. The pots are then kept permanently in the glasshouse until completion of the experiment. Two undamaged fully expanded quadrifoliate leaves of the main axis of a plant per pot, five plants per replication for each genotype, are tagged to study the components of resistance (Dwivedi et al. 2002a). The experiment should be terminated at 45 to 50 d after inoculation or when the disease is fully developed and most of the leaves are either completely weathered due to rust or defoliated due to leaf spots.

Reports on the genetic basis of resistance to rust and leaf spots vary from a few genes to quantitative inheritance (reviewed by Dwivedi et al. 2003a). Resistance to rust is due to longer incubation and latent periods, fewer pustules per leaf, smaller pustule diameter, lower sporulation index, less leaf area damage, and lower disease score. Since these components interact in an additive manner, selection is based on evaluation of individual components of resistance together with the determination of green leaf area remaining on the plant. Resistance to LLS is due to longer incubation and latent periods, fewer lesions per leaf, smaller lesion diameter, lower sporulation index, less leaf area damage, and lower disease score. Selection based on components of resistance to LLS may not lead to plants with higher retained green leaf area. The remaining green leaf area on the plant and disease score should be the basis of selecting for resistance to LLS (Dwivedi et al. 2002a). The ELS-resistant germplasm accessions have longer incubation periods, reduced sporulation rates, lesion diameter, and infection frequencies, and less defoliation (Nevil 1981; Waliyar et al. 1993).

Aflatoxin

Aflatoxins are produced by *Aspergillus flavus* Link ex Fries, which produces only aflatoxin B₁. Resistance to *A. flavus* in peanut operates independently in at least three tissues: pod, seed coat, and cotyledons (Mixon 1986). Resistance to pod infection is attributed to pod-shell structure, while resistance to seed invasion and colonization has been correlated with thickness, density of palisade cell layers, absence of fissures and cavities, and presence of wax layers

on the seed coat. A number of genes are involved in seed response to colonization by *A. flavus*, aflatoxin production, and preharvest infection (Utomo et al. 1990). Estimates of broad sense heritability vary from low to high for natural infection and seed colonization by *A. flavus* and from low to moderate for aflatoxin production (http://www.aflatoxin.info/groundnut_breeding.asp).

Three-phase evaluation is recommended for phenotyping of the parental and mapping populations: preharvest infection (Mehan 1989), in vitro seed colonization (Mixon and Rogers 1973; Mehan and McDonald 1980), and aflatoxin production (Mehan and McDonald 1980). Drought during pod formation substantially increases the level of aflatoxin contamination. Preharvest infection requires a drought period of 30 to 35 d and a mean soil temperature of 29 to 31 °C in the podding zone (Cole et al. 1989, 1995). The susceptibility of peanut to aflatoxin contamination is related to lower water activity (0.80 to 0.95) in the kernel and favorable temperature (25 to 32 °C) for growth of *A. flavus* (Sicherer et al. 1999). As the kernel moisture content decreases under end-of-season drought, protection from the natural defense mechanisms is lost and the kernels become vulnerable to colonization by *A. flavus* and aflatoxin contamination. A line-source sprinkler irrigation system (Hanks et al. 1976) that imposes a water deficit gradient is suggested to evaluate populations for their reaction to *A. flavus* and aflatoxin production. The most common immunoassay used for mycotoxin detection are enzyme-linked immunosorbent assay (ELISA); analyzing a single analyte by ELISA costs ca. 5.9% and 2.5% of that by gas chromatography or gas chromatography-mass spectroscopy, respectively (Vogt 1984). Chu et al. (1987) developed an improved ELISA technique that is more sensitive than previously reported methods (El-Nakib et al. 1981; Ram et al. 1986a,b) and analytical time shortened to less than 1 h for quantitative analysis and less than 30 min for screening tests, with a detection power as little as 5 pg B₁. A few commercial kits available in the market are Aflatest, Agriscreen, AflaCup 10, AflaCup 20, and EZ-Screen, with 1 to 20 ppb detection power; approximate cost per sample ranges from US\$ 8.5 to 65.0 for quantitative estimates and US\$ 6.0 to 7.5 for visual tests (www.oznet.ksu.edu/grsiest).

Allergens

ELISA is the most commonly used method to detect peanut in the food manufacturing process and in

food products (Holzhauser and Vieths 1999), whereas peanut allergens are measured in foods by immunoassays with human IgE antibodies (Keating et al. 1990). However, these assays do not provide quantitative measurements of exposure to food allergens. Hird et al. (2003) developed a sensitive and robust assay for the identification of peanut in commercial products using real-time PCR technology, while Pomes et al. (2003) developed a new sensitive and specific monoclonal antibody-based ELISA to monitor Ara h1 content in food products and in developing thresholds for sensitization or allergic reaction in persons with peanut allergy.

Dodo et al. (2002) provided a detailed procedure to detect peanut allergens that consists of defatting peanut seeds, extracting peanut proteins, and conducting an ELISA test.

Nematodes

The peanut root-knot nematode *Meloidogyne arenaria* (Neal) Chitwood race 1 is widely distributed across peanut-growing areas. Holbrook et al. (1983) described the screening technique wherein greenhouse-grown plants are inoculated with 3000 eggs of *M. arenaria* race 1 cultured on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) or eggplant (*Solanum melongena* L. cv. Black Beauty). The nematode inoculum should be prepared using the NaOCl method (Hussey and Barker 1973) and applied 14 d after planting. Approximately 70 d after inoculation, plants are uprooted and washed clean of soil. The roots are then placed in 1000-ml beakers containing 300 ml of 0.05% ploxine B solution for 3 to 5 min (Daykin and Hussey 1985) and scored for root galls and egg masses [using dissecting microscope (×20) to determine the number of eggs per gram of fresh root weight] based on a 0 to 5 rating where 0 = no galls or no egg masses, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, and 5 = more than 100 galls or egg masses per root system (Taylor and Sasser 1978). Some variation in screening procedure may be adapted as reported in other publications (Nelson et al. 1989; Starr et al. 1995; Choi et al. 1999).

Resistance to *M. arenaria* is moderate in cultivated peanut and is due to reduction in the percentage of second-stage juveniles (J2) that establish a functional feeding site (Timper et al. 2000). In contrast to cultivated peanut, very high levels of resistance to *M. arenaria* exist in wild *Arachis* species conditioned by

two dominant genes: *Mag* inhibits root galling and *Mae* inhibits egg production by *M. arenaria* (Garcia et al. 1996).

Defoliating Insect Pests

The major defoliators in peanut are leaf miner [*Aproaerema modicella* (Deventer)] and tobacco armyworm (*Spodoptera litura* F.). It is difficult to screen for resistance to defoliators under natural field infestation because of variation in infestation in space and time. Sharma et al. (2002) developed a no-choice cage technique to screen for resistance to *S. litura*. Essentially the technique consists of raising the nuclear insect cultures and maintaining these on an artificial diet (Taneja and Leuschner 1985), releasing a known number of first- or third-instar larvae on 15-d-old greenhouse plants (temperature $28 \pm 5^\circ\text{C}$ and RH > 65%) grown in pots (30 cm diameter, 30 cm deep) under a plastic jar cage (11 cm diameter and 26 cm height) with two wire-mesh-screened windows (4 cm diameter) for varying periods of time and then recording observations on insect survival and leaf area damage. Two plants are grown in each pot, where one plant is infested with the larvae inside the cage and the other plant remains outside the cage and is left as an uninfested control. Observations are then recorded on the number of surviving larvae, the larval weight (4 h following termination of the experiment), and percentage leaf area damage on a 1 to 9 scale, where 1 = 10% leaf area damage, 2 = 11 to 20%, 3 = 21 to 30%, 4 = 31 to 40%, 5 = 41 to 50%, 6 = 51 to 60%, 7 = 61 to 70%, 8 = 71 to 80%, and 9 > 80% leaf area damaged.

At present screening for resistance to leaf miner is dependent on natural infestation under field conditions as it is a difficult pest to devise a cage-based greenhouse screening procedure for. However, prolonged drought and planting soybean as infester crop favor high intensity of leaf miner infestation under field conditions. A 1 to 9 scale, similar to that used for screening resistance to *Spodoptera*, is suggested to record percentage leaf area damage by leaf miner.

A high level of tolerance to leaf miner and *Spodoptera* has been observed in breeding line ICGV 86031 and is manifested as the enhanced ability of the vegetative tissue to regrow subsequent defoliation (Wightman and Rao 1994). Several wild *Arachis* species have also shown resistance to leaf miner and *Spodoptera* with morphological traits such as main stem thickness, hypanthium length, leaflet shape

and length, leaf hairiness, standard petal length and petal markings, basal leaflet width, and main stem thickness and hairiness, and stipule adnation length and width showed significant correlation and/or regression coefficients with damage by defoliators including *S. litura* (Sharma et al. 2003).

Groundnut Rosette Virus

The rosette (chlorotic and green) is the most destructive disease of peanut in sub-Saharan Africa. Three agents interact to produce rosette disease syndrome: groundnut rosette virus (GRV), groundnut rosette assistor virus (GRAV), and satellite RNA (sat RNA) (Bock et al. 1990). GRV is transmitted by aphid (*Aphis craccivora*) but only from the plants that also contain GRAV. GRAV is not mechanically transmissible and causes no apparent symptoms in peanut. The sat RNA, which is dependent on GRV for multiplication and on GRAV for aphid transmission, is largely responsible for rosette symptoms (Murant et al. 1988). Variation in sat RNA has been correlated with the different forms of rosette disease (Murant and Kumar 1990). All three agents must be present together in the host plant for successful transmission of the disease by the aphid vector.

Bock and Nigam (1988) developed the infector-row technique where a highly susceptible peanut cultivar is grown in every two rows of the test materials under field conditions. Potted spreader plants of the highly susceptible cultivar showing severe rosette symptoms and heavily infested with aphids are raised in a glasshouse and transplanted to the infector rows (1 plant per 3 m rows) 10 d after sowing the test materials. The disease development in the infector rows is monitored ca. 2 weeks later, and viruliferous aphids are released onto the plants that are free from the disease. The disease incidence is then assessed at the pod-filling stage. The percentage of disease incidence is determined based on the total number of plants per plot and the number of plants showing rosette symptoms with severe stunting; using a 1 to 3 scale for classifying the disease reaction where 1 = plants with no symptoms on foliage and no stunting, 2 = plants with obvious rosette leaf symptoms and stunted to about 50% of the size of the normal plants, 3 = plants with severe rosette leaf symptoms and stunting greater than 50% (Olorunju et al. 1991) with some modification (Subrahmanyam et al. 1998). The disease index is then calculated based on this rating as $(A + 2B + 3C)/\text{total}$

number of plants assessed per plant, where A, B, and C equal the number of plants with ratings of 1, 2, and 3, respectively.

The selected lines from field trials should be evaluated (50 to 60 d after aphid inoculations) for the presence of GRAV using TAS-ELISA (Rajeshwari et al. 1987). GRV and sat RNA are detected by RT-PCR as described by Naidu et al. (1998), and a field and greenhouse evaluation is recommended to screen for resistance to aphid (Padagham et al. 1990).

Resistance to GRV virus has been reported to vary from monogenic dominance to two independent recessive genes (Nigam and Bock 1990; Olorunju et al. 1992), while aphid resistance may be controlled by a single recessive gene (Herselman et al. 2004).

Peanut Clump Virus

Viruses of the genus *Pecluvirus* cause peanut clump disease (Torrance and Mayo 1997), referred to as peanut clump virus (PCV) in West Africa and Indian peanut clump virus (IPCV) in South Asia. The fungus *Polymyxa* spp transmits IPCV (Ratna et al. 1991) and PCV is suspected to have the same vector. Both IPCV and PCV have an extremely wide host range including many monocots (Ratna et al. 1991; Delfosse et al. 1996), are soilborne, and produce similar symptoms on peanut. However, IPCV is not serologically related to two West African isolates (Reddy et al. 1983).

Severely stunted plants with dark green leaves and mosaic mottling with chlorotic rings on new quadrifoliate characterize the peanut clump disease in peanut (Reddy et al. 1983). The disease occurs in patches in the field, which reappears in the same positions in the following season, indicating high resilience but slow movement of the vector. A field with a known history of high disease incidence should be selected for screening for resistance to peanut clump disease. An ELISA and nucleic acid hybridization test is recommended to confirm the presence of the virus in the infected plants from the field (Delfosse et al. 1999). The intensity of disease incidence is measured by visual symptoms and ELISA test.

Drought

Genotypes are usually evaluated for drought tolerance under field conditions during the dry season with controlled supplementary irrigation. However, it is difficult to fully represent the natural drought environment where moisture stress occurs during the

rainy season in which the crop is largely grown by the farmers. Thus, it is recommended that rainout shelters be created for drought stress screening during the rainy season, but this does require considerable investment. Crop phenology is the single most important trait for enhancing performance under drought stress. Fortunately, peanut is temperature sensitive but not daylight sensitive like many crops, so its phenology is not especially driven by the environment. Specht et al. (1986) defined drought tolerance in three general categories: (a) escape for drought by tailoring plants with appropriate phenologies to fit the most appropriate growing period, (b) dehydration avoidance through identification and incorporation of traits that lessen evaporatory water loss or increase water uptake through deeper and more extensive root system, and (c) dehydration tolerance by selecting traits that maintain cell turgor (a driving force for plant growth) or enhancing cellular constituents that protect cytoplasmic proteins and membranes from desiccation. In contrast, empirical or yield-based definitions of drought tolerance fall into two categories: (i) absolute, where breeders select for the highest yielding genotypes in environments where seasonal drought is predictably recurrent, and (ii) relative, where breeders select for genotypes with the smallest yield decline per unit of reduced seasonal rainfall (Specht et al. 1986).

Three profiles of drought stress can be differentiated for all crops including peanut – (i) early-season drought, (ii) midseason drought, and (iii) end-of-season (terminal) drought – and the effect of these on yield depends on the severity and duration of water deficit stress and the drought management practices applied. Moderate early drought stress is actually beneficial for the establishment of the peanut crop, while midseason and terminal drought can cause substantial reduction in peanut yield (Nageswara Rao et al. 1989). To impose midseason drought stress in peanut, the irrigation is withheld from 40 d after sowing (DAS) to 80 DAS, and then the stress is released (by irrigating the crop on a regular basis until maturity) to measure the recovery response of the stressed genotypes. These 40 d of stress coincide with flowering and early pod development in peanut. For end-of-season drought, the irrigation is withheld from 80 DAS until the crop is harvested.

The line-source sprinkler technique (Hanks et al. 1976) is used to create a gradient of irrigation across contiguous plots. The plot nearest to the sprinkler head receives ca. 50 mm of water, thus providing an

irrigated control. The amount of water then decreases in a linear fashion as the distance of the plot from the sprinkler head increases.

Several sources of tolerance to midseason and/or terminal drought have been reported in peanut (Nageswara Rao et al. 1989; Nigam et al. 2003a,b) that showed variation for physiological traits such as specific leaf area (SLA), water use efficiency (WUE), amount of water transpired (T), transpiration efficiency (TE), and harvest index (HI) under drought stress conditions (Nageswara Rao et al. 1993; Nageswara Rao and Wright 1994; Wright et al. 1994, 1996; Craufurd et al. 1999; Nageswara Rao and Nigam 2001). The component trait-based approach based on assessing variation in SLA, WUE, T, TE, and HI under drought stress conditions is recommended for accurate and precise dissection of drought tolerance in peanut. Variation in WUE arises mainly from genotypic differences in water use. Carbon isotope discrimination (Δ) can be used to select genotypes with improved WUE under drought conditions in the field. A strong relationship between WUE and SLA and between Δ and SLA revealed that genotypes with thick leaves had greater WUE (Wright et al. 1994). SLA could therefore be used as a rapid and inexpensive indirect selection criterion for WUE to facilitate selection for terminal drought tolerant genotypes (Nageswara Rao and Wright 1994). However, there appears to be a negative relationship between WUE and HI under terminal drought stress conditions, suggesting that selection for high WUE might enhance dry matter production under stress but not necessarily improve pod yield (Nageswara Rao and Wright 1994; Wright et al. 1994). SLA is also highly influenced by $G \times E$ interaction. Nageswara Rao et al. (2001) demonstrated the use of a portable SPAD chlorophyll meter for rapid assessment of SLA and specific leaf nitrogen (SLN) as surrogate traits to measure TE in peanut.

Both additive and additive \times additive epistasis for SLA and HI and additive genetic effect for Δ are reported (Jayalakshmi et al. 1999; Nigam et al. 2001).

Seed Quality

Total oil and protein content and fatty acid profile are the important seed quality traits that substantially influence the edible uses of peanut. Near-infrared (NIR) transmittance spectroscopy (Panford 1990; Misra et al. 2000) and nuclear magnetic resonance

(NMR) spectrometer (Jambunathan et al. 1985) is preferred over the conventional Soxhlet (Kuck and St Angelo 1980) method for determining the oil content as these tests are rapid (2 to 3 min), do not require any extraction reagents or supporting equipment, and the seeds can be used after the test. However, NIR requires a larger sample (150 g) over NMR (3 to 5 g) but can also provide additional information on protein content and other quality parameters. A Technicon autoanalyzer may also be used to determine nitrogen concentration (Singh and Jambunathan 1980) and then multiply the value by a factor of 5.46 to convert nitrogen into crude protein content (United Nations University 1980).

Fatty acid composition is determined following Hovis et al. (1979), and from it the following quality parameters can be determined (Mozingo et al. 1988).

1. Iodine value (IV): (% oleic acid) (0.8601) + (% linoleic acid) (1.7321) + (% eicosenoic acid) (0.7854)
2. Oleic (O)/linoleic (L) acid ratio = % oleic acid/% linoleic acid
3. Total saturated fatty acids (%) (TSF) = % palmitic acid + % stearic acid + % arachidic acid + % behenic acid + % lignoceric acid
4. Polyunsaturated (P)/saturated (S) ratio = % linoleic acid/% TSF
5. Total long-chain saturated fatty acids (%) (TLCSF) = % arachidic acid + % behenic acid + % lignoceric acid

3.3.2

Germplasm with Beneficial Traits for Mapping and Genetic Enhancement

A large number of accessions possessing resistance/tolerance to abiotic and biotic stresses have been identified both in cultivated and wild *Arachis* species (Table 2). Of these, a number of promising accessions/breeding lines, mostly based on genetic diversity, differing in resistance/tolerance mechanism to biotic and abiotic stresses, or varying in seed quality, including peanut allergens have been recommended for use in mapping and genetic enhancement in peanut (Table 3).

Table 3. List of potential germplasm/breeding lines resistant to rust, leaf spots, aflatoxin, rosette, nematode, defoliators, aphid, drought, low temperature, and those with improved agronomic traits, oil chemistry and low allergens for mapping and genetic enhancement in cultivated peanut

Trait	Identity	Reference
Rust	ICGV# 99003 and ICGV 99005	Dwivedi et al. 2002a; ICRISAT 2004
Leaf spots	ICG # 405, 1705, 6284, 9987, 9991, 9989, 10000, and 10914; ICGV# 99001 and 99104; PI 565287 (TxAG-6) and PI 565288 (TxAG-7)	Dwivedi et al. 2002a; Dwivedi and Gurtu 2002; Simpson et al. 1993; ICRISAT 2004
Groundnut rosette virus	ICG#3436, 6323, 7827, 9558, 11044, and 11968	Dwivedi et al. 2003a
Nematode	PI 565287 (TxAG-6) and PI 565288(TxAG-7)	Simpson et al. 1993
Seed infection and/or aflatoxin production by <i>Aspergillus flavus</i>	ICG# 1326, 1448, 1471, 4681, 4749, and 7101 ICGV 88145 and ICGV 89104 ICGV 91278, ICGV 91279, and ICGV 91283	Dwivedi et al. 2001; Mehan et al. 1985 Rao et al. 1995 Upadhyaya et al. 2001c
Defoliators (Leaf miner and <i>Spodoptera</i>)	ICGV# 86031, 87154 and 87160; ICG# 2271 and 1697 + from GP-NCWS7, GP-NCWS8, GP-NCWS9, and GP-NCWS10	Dwivedi et al. 1993; Wightman and Rao 1994 Stalker and Lynch 2002
Aphid	ICG 12991	Herselman et al. 2004
Drought	ICG 1471; ICGV# 94106, 94113, 96294, 97068, 97093, 98381, 98382, 99231; 99233, 99235, 99236; 99237, 99238, 99241, 99243, 99247; 99249, and 99255; and CSMG 84-1 ICG# 766 and 14523; ICGV 86031 and TAG 24 ICG# 118, 532, 862, 2106, 2511, 2773, 4527, 5236, 5827, 6654, 6766, 7243, 8285, 11219, 11855, 14475, 14523, 14985	Dwivedi and Varma 2002 ICRISAT 2004 Upadhyaya 2005
Oleic/linoleic fatty acid ratio	UF 435-2-1, and UF 435-2-2	Norden et al. 1987
Allergen	PI 261924 and PI 338386	Dodo et al. 2002
Early maturity	ICG # 3200, 3540, 3631, 4558, 4729, 4890, 5512, 5560, 5881, 9427, 9930, 9968, 11605, 11914, 13585, 13606, 13647, 14390, 14788, 14814, 14815	Upadhyaya et al. 2006
Low temperature	158 accessions ICGV 92267	ICRISAT unpubl. data Upadhyaya et al. 2002b
Yield and agronomic traits	60 accessions	Upadhyaya et al. 2005

3.4

Genomic Resources in Peanut

3.4.1

DNA Markers (RFLPs, RAPDs, AFLPs, SSRs)

Early studies on peanut genomics focused on screening cultivated peanut germplasm and/or tetraploid interspecific breeding lines with 67 polymorphic RAPD markers (Burow et al. 1996; Bhagwat et al. 1997; Choi et al. 1999; Subramanian et al. 2000; Raina et al. 2001; Dwivedi et al. 2001, 2002b) and 404 polymorphic RFLP markers (Garcia et al. 1995; Burow et al. 2001). However, these marker assays are not ideal for use in MAS. RFLP, although providing high-quality codominant information, is labor intensive and time consuming, requires large amount of DNA, and is dependent on radioisotope-based protocols, while RAPD analysis provides only dominant information and frequently suffers from reproducibility problems. However, it is possible to convert tightly linked RFLP markers into PCR-based sequence-tagged site (STS) markers (Olson et al. 1989) and similarly to convert RAPD bands into sequence-characterized amplified region (SCAR) markers (Paran and Michelmore 1993). STS and SCAR assays provide substantially more reliable markers with a relative high-throughput potential. Most of the genetically mapped RFLPs have also been sequenced. Like RAPDs, AFLPs are also a dominant marker class but can be converted into codominant marker such as SCAR (Paran and Michelmore 1993; Negi et al. 2000; Huaracha et al. 2004) and CAPS (Konieczny and Ausubel 1993). Forty-five of the 64 *EcoRI/MseI* primer pairs were polymorphic in cultivated peanut germplasm/cultivars and interspecific derivatives (Table 4). Herselman (2003) used two different rare cutters enzymes, *EcoRI* and *MluI*, in combination with the frequent cutter *MseI*, and found that both *EcoRI/MseI* and *MluI/MseI* AFLP enzyme combinations efficiently detected polymorphisms within closely related cultivated peanut accessions, although the *EcoRI/MseI* enzyme combination detected more fragments per primer combination (on average 67.8) as opposed to 29.1, by the *MluI/MseI* enzyme combination on the similar peanut accessions.

Simple sequence repeat (SSR, also known as microsatellites) markers have become the assay of choice

for molecular breeding of most crops. SSR markers are valuable for a multitude of applications due to their abundance and uniformity of distribution throughout most genomes, their multiallelic, codominance inheritance, and their highly polymorphic and reproducible nature where analysis is simple and readily transferable (Weber 1990). Hopkins et al. (1999) were the first to report six polymorphic SSR markers in cultivated peanut. Further search for SSRs in peanut led to the development of 553 SSRs of which 192 SSRs were polymorphic in a diverse range of cultivated peanut accessions (Table 5). Moretzsohn et al. (2004) reported high marker transferability for markers from species related to peanut: up to 76% from species of the section *Arachis* and up to 45% from species of the other eight *Arachis* sections. Similarly, efforts at ICRISAT have supported this finding (Mace et al. unpubl. data). However, there appears to be a high level of redundancy in this approach, such that, although SSR markers from related species and genera do amplify in *Arachis* germplasm, few are found to be polymorphic in groundnut breeding populations.

Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) and Universidade Catolica de Brasilia (UCB), Brazil, have recently put considerable effort into large-scale development of additional SSR markers. Genomic libraries enriched for TC and AC repeats (Rafalski et al. 1996) have yielded 126 new SSR markers. To facilitate the selection of SSR-containing sequences, and the assembly of forward and reverse sequencing runs, a dedicated module (available from David Bertoli on request) has been developed for the Staden sequence assembly software (Staden et al. 2003a,b). In addition to the enriched genomic libraries, the module has also been used to "data-mine" 31 EST-SSR markers from data available in Genbank and 117 EST-SSR markers from *A. stenosperma* EST data.

Similar efforts have also been made by ICRISAT through the library enrichment process. Based on these recent marker development projects, groundnut is now one of the best-served tropical legumes in terms of SSR markers for linkage mapping and molecular breeding (Table 5). However, it is notable that these recent advances have been driven largely by labs based in developing countries rather than by advanced labs in Western countries that have traditionally fulfilled this role. The availability of such a large number of AFLPs and SSRs markers holds great promise for diversity studies,

Table 4. Polymorphic AFLP primer pairs reported in cultivated peanut accessions

AFLP primer	Herselman 2003	Gimenes et al. 2002	Dwivedi et al. 2002b	He and Prakash 2001	He and Prakash 1997
E-AAC/M-CAT					
E-AAC/M-CTA	*		*		
E-AAC/M-CTC	*				
E-AAC/M-CTG	*		*		
E-AAC/M-CTT	*		*		
E-AAC/M-CAA				*	*
E-AAC/M-CAC			*		
E-AAC/M-CAG				*	*
E-AAG/M-CAC	*			*	*
E-AAG/M-CAG	*			*	*
E-AAG/M-CAT	*				
E-AAG/M-CTT					*
E-ACC/M-CAA	*				
E-ACC/M-CTG		*			
E-ACC/M-CTC			*		
E-ACC/M-CAT			*		
E-ACC/M-CAC			*	*	*
E-ACC/M-CAG				*	*
E-ACC/M-CTA				*	*
E-ACG/M-CAC	*				*
E-ACG/M-CTA	*				
E-ACG/M-CAT				*	*
E-ACG/M-CTG				*	*
E-ACG/M-CTT				*	*
E-ACG/M-CAG				*	*
E-ACG/M-CAA					*
E-ACG/M-CTC					*
E-ACT/M-CAA	*			*	*
E-ACT/M-CAT	*		*		
E-ACT/M-CTA	*			*	*
E-ACT/M-CTC	*				
E-ACT/M-CTG	*				
E-ACT/M-CTT		*			*
E-ACT/M-CAG					*
E-AGC/M-CAA	*		*		
E-AGC/M-CTG		*			
E-AGC/M-CTT				*	*
E-AGG/M-CAA	*				*
E-AGG/M-CAC	*				*
E-AGG/M-CAT	*				*
E-AGG/M-CTA	*		*		*
E-AGG/M-CTT			*		*
E-AGG/M-CAG			*		*
E-ACA/M-CAG			*		
E-ACA/M-CTC			*		
E-ACA/M-CTG			*		*
E-ACA/M-CAA				*	*

Table 5. List of total number of SSRs and those polymorphic in cultivated peanut accessions

Source and method of isolation	Total number	Most frequently repeat	Results from screening cultivated germplasm		Reference
			Germplasm represented	Number detecting polymorphism	
cDNA libraries from peanut cultivar Florunner and New Mexico Valencia	26	GA	19	5	Hopkins et al. 1999
SSR enrichment procedure (Edwards et al. 1996)	56	GA CT	24	19	He et al. 2003
cDNA libraries from peanut cultivar Florunner	226	ATT GA	24	110	Ferguson et al. 2004a
Rafalski et al. (1996)	67	TTG AAC	60 cultivated peanut accessions	3	Moretzsohn et al. 2004
SSR enrichment procedure (Rafalski et al. 1996)	122	TC AC	16 cultivated peanut accessions	53	EMBRAPA (unpub)
EMBRAPA ESTs and Genbank deposited sequences	112	AAG AAT CT	16 cultivated peanut accessions	9	EMBRAPA (unpub)
Data-mining other sequences	38	CT AG	16 cultivated peanut accessions	5	EMBRAPA (unpub)
Edwards enrichment process (Edwards et al. 1996)	125	GT CA	32 diverse genotypes include 24 from Ferguson et al. 2004b	55	Tho et al. unpub data (ICRISAT)

genetic mapping and MAS, and gene discovery in peanut.

3.4.2 Molecular Diversity

Diversity assessment and construction of genetic linkage maps are the two important steps in the development of molecular breeding programs. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, hosts the world collection of 14,966 accessions of the cultivated and 453 of wild *Arachis* species. These accessions differ in many morphophysiological, reproductive, and quality traits, and in response to biotic and abiotic stresses (Rajgopal et al. 1997; Singh and Nigam 1997; Upadhyaya et al. 2001a, 2003). For enhancing the use of peanut germplasm in breeding, two-core and minicore collections (ICRISAT core of 1,704 and minicore of 184 accessions and USDA core of 831 accessions and core of core 111 accessions) are reported in peanut. The accessions included in the core and minicores have the potential to identify new sources of variation for use in peanut genomics and breeding (see Sect. 2.3. for further details). The minicore is good starting material for association mapping and for the detection of rare allelic variation associated with beneficial traits.

In contrast with the historical generalization that cultivated peanut lacks genetic variation (Grieshammer and Wynne 1990; Kochert et al. 1991; Bhagwat et al. 1997; He and Prakash 1997; Subramanian et al. 2000), genetic diversity studies in the last few years have revealed sufficient polymorphic variations among cultivated peanut germplasms that could be tapped to identify markers associated with beneficial traits and possibly effect marker-assisted genetic enhancement in peanut (Hopkins et al. 1999; Dwivedi et al. 2001; He and Prakash 2001; Raina et al. 2001; Dwivedi et al. 2002b; Dwivedi and Gurtu 2002; Dwivedi and Varma 2002; Gimenes et al. 2002; Dwivedi et al. 2003a,b; Herselman 2003; Krishna et al. 2003; Ferguson et al. 2004b; Moretzsohn et al. 2004). Both AFLP and SSR are useful for estimating diversity among the *Arachis* species and six botanical types of cultivated peanut (He and Prakash 2001; Gimenes et al. 2002; Herselman 2003; Ferguson et al. 2004b; Moretzsohn et al. 2004).

Most recently, ICRISAT has been using SSR markers to analyze the genetic diversity among cultivated germplasm resistant to late leaf spot (LLS), rust, and

bacterial wilt (Mace et al. unpubl. data). In these studies, SSR markers were able to detect a surprisingly high level of polymorphism. In some cases, more than half of the markers detected polymorphism with PIC values of over 0.5. This has also opened the possibility of following association mapping in cultivated peanut germplasm for the identification of markers for disease resistance.

Although recent studies on genetic diversity revealed polymorphisms in cultivated peanut, the levels of polymorphism detected are still low for easy construction of saturated maps. In contrast, much higher polymorphism is reported in wild *Arachis* species. For example, only three of the 67 SSRs were polymorphic on 60 cultivated accessions belonging to 6 botanical varieties, whereas 28 were polymorphic on two wild *Arachis* accessions (*A. duranensis* K7988 and *A. stenosperma* V10309) (Moretzsohn et al. 2004) used to make the diploid AA genome mapping population (see Sect. 4.3. for further details). This higher level of polymorphism greatly facilitates genetic mapping. Conventionally, the use of interspecific mapping populations has not been encouraged by plant breeders because of the divergent recombination patterns evident in such populations as compared with the intraspecific breeding populations where resultant markers would be applied. This divergence often leads to a loss of selective power of the marker. However, peanut is an amphiploid, viz. an allotetraploid with two different genomes, that behaves genetically as if two separate diploids are in the same cell. Thus, the application of diploid maps may be much more directly applicable and effective than conventional interspecific mapping populations.

3.4.3 Mapping Population

Near-isogenic lines (NILs) (Muehlbauer et al. 1988), recombinant inbred lines (RILs) (Burr et al. 1988), doubled-haploid (DH) populations (Heun 1992), and advanced backcross-derived RILs are the preferred types of mapping populations in plant genomic studies as these are immortal genetic stocks (unlike F_2) that can be recurrently tested in replicated trials across locations and seasons. Tanksley and Nelson (1996) have proposed advanced backcross lines for the simultaneous discovery and transfer of valuable QTLs from unadapted and wild germplasm into elite breeding lines. Similarly, Podlich et al. (2004) have proposed

a refinement and expansion of this type of approach. This approach acknowledges that the size of mapping populations and the presence of a consistently adapted agronomic background across all members of that population are critically important factors for the precise and accurate mapping of economically important characters, particularly complex traits. A population of 250 to 300 F_2 derived RILs should be sufficient to detect the chromosomal region (more precisely the QTL location) associated with most beneficial traits. The small population sizes all too often used for QTL detection lead to overestimation of QTL effect and underestimation of QTL number and interaction due to what is now commonly referred to as the "Beavis Effect" (Beavis 1998; Melchinger et al. 1998).

Efforts are being made by ICRISAT and EMBRAPA to develop mapping populations involving *A. hypogaea* \times *A. hypogaea*, *A. hypogaea* \times wild *Arachis*, and wild *Arachis* \times wild *Arachis* species crosses. RIL mapping populations are now available for rust, LLS, and drought for evaluation, and seeds from these populations are available upon request under the respective institutional material transfer agreement.

Recently, EMBRAPA-CENARGEN (EMBRAPA Recursos Genéticos e Biotecnologia), UCB, and UNESP (Universidade Estadual de São Paulo)-Botucatu (Brazil) have developed diploid mapping populations from crosses of wild *Arachis*. F_2 populations have been made that represent the AA and BB genomes of cultivated peanut, *A. duranensis* and *A. ipaensis* crossed with closely related species *A. stenosperma* and *A. magna*, respectively. The original hybrids are being maintained and the F_2 plants multiplied by cuttings. Currently the mapping populations are of 93 plants that are being advanced to develop RILs. Through this strategy, consensus maps will be created by comparative analysis of diploid and tetraploid maps. These diploid maps will facilitate the marker-assisted introgression of a wide range of important agronomic traits into cultivated peanut. In addition, a tetraploid mapping population has been developed from the cross of a synthetic amphidiploid (*A. duranensis* \times *A. ipaensis*)^c with cultivated *A. hypogaea*.

3.4.4

Genetic Linkage Map

High-density genetic linkage maps are a useful basis for identifying markers tightly linked to QTLs that contribute to economically important traits (Pa-

terson et al. 1988; Lander and Botstein 1989), for cloning genes by chromosome walking (Wicking and Williamson 1991), and for developing MAS systems for desirable traits in breeding programs (Burr et al. 1983; Tanksley et al. 1989).

Cultivated peanut is a disomic polyploid ($2n = 4x = 40$); thus in genetic maps we expect 20 linkage groups (LGs), each representing one haploid complement chromosome. Halward et al. (1993) developed the first low-density RFLP-based genetic linkage map in peanut, derived from an interspecific F_2 population involving A genome diploid species *A. stenosperma* and *A. cardenasii*. This map comprised 117 markers on 11 LGs with a total map distance of ca. 1,063 cM and average marker density of 9.08 cM. Subsequently, Burow et al. (2001) reported an RFLP-based tetraploid genetic linkage map derived from a BC_1 population ($n = 78$) from the cross: synthetic amphidiploid {[*A. batizocoi* K9484 \times (*A. cardenasii* GK10017 \times *A. digoi* GKP10602)]^{4x}} crossed with cv. Florunner. *A. cardenasii* and *A. digoi* are A genome while *A. batizocoi* belongs to B genome species. This map consists of 370 RFLP loci distributed into 23 LGs with a total map distance of 2,210 cM and average marker density of 5.97 cM. These RFLP loci will detect alleles in populations involving crosses between wild species or between *A. hypogaea* \times wild *Arachis* species. They are unlikely to detect alleles in *A. hypogaea* \times *A. hypogaea* crosses.

In the last few years, there has been substantial progress on identifying polymorphic AFLPs and SSR markers (see sections 4.1 and 4.2). A partial AFLP-based genetic linkage map, based on an intraspecific *A. hypogaea* cross, has been developed for mapping aphid resistance that mapped 12 markers to five LGs covering a map distance of 139.4 cM (Herselman et al. 2004). Significant progress has also been made at ICRISAT in the mapping of disease resistances using AFLP, SSR, and RGA markers (Mace et al. unpubl. data). Preliminary maps comprise around 75 markers across 16 LGs covering a map length of 423 cM (rust resistance) and around 70 markers across 9 LGs covering a map length of 175 cM (LLS resistance). A skeleton map has also been generated for resistance to ELS, and mapping of bacterial wilt resistance is ongoing. As new SSR markers are becoming available, they are being integrated into these maps in order to increase the total map length and marker density.

The new SSR markers developed at EMBRAPA/UCB and ICRISAT are being used for the development of diploid maps of the A and B genomes.

To date, an SSR-based linkage map of the AA genome of *Arachis* contains 153 SSR markers mapped on 11 LGs with a total map distance of 1,138.39 cM and 7.43 cM average marker density. In addition, a BB-genome linkage map is being made in collaboration with UNESP-Botucatu. It is anticipated that these diploid linkage maps will enable mapping of some markers and traits that would be difficult to deal with in a tetraploid background. Diploid maps will then be verified using a tetraploid mapping population based on an F₂ population derived from a cross between a synthetic amphidiploid (*A. duranensis* × *A. ipaensis*)^c and *A. hypogaea*.

3.4.5

Comparative Mapping with Model Genomes

An ongoing project between Aarhus University, Denmark, EMBRAPA, and UCB Brazil aims to integrate *Arachis* into a single unified legume genetic framework using "legume family anchor markers" for legumes. These are gene-based markers with a single homolog representation in the *Arabidopsis* proteome that are being mapped in *Lotus japonicus*, lupin, bean, and the diploid AA genome of *Arachis*.

To efficiently identify potential anchor marker sequences, a computer "pipeline" that uses multispecies EST- and genome-sequence data has been developed. Comparison of ESTs from *Medicago truncatula*, *Glycine max*, and *L. japonicus* identifies evolutionary conserved sequences (ECSs) that have a high probability of being conserved in less well-characterized legumes. Alignment of ECSs and a corresponding genomic sequence defines sets of PCR primer sites flanking introns. Introns are targeted because purifying selection is less stringent for coding regions, and they are more likely to be polymorphic. The length of introns is important because short introns are less likely to be polymorphic than longer ones and because the final PCR reaction is limited to a maximum amplicon size of ~2.5 kb using standard polymerases. Finally, only marker sequences with single homologs in the *Arabidopsis* proteome are selected for further development. Using this approach, 867 ECSs have been identified, and these are being used for marker development. Polymorphisms are identified by size or sequence differences in PCR products, amplified from mapping parents, and CAPs or dCAPs markers developed. So far, 40 markers have been developed for

the diploid AA genome *Arachis* mapping population (David Bertoli, unpubl. data).

Comparing the map positions of ECS markers in different legumes should allow the development of a preliminary comparative map across legume crops and model systems. The inclusion of *Arachis* within this analysis is likely to be especially informative because *Arachis* together with lupin occupies a basal phylogenetic position within the Papilionoideae. This work will then enable model organisms with well-characterized genomes to serve as genic frameworks for the poorly characterized *Arachis* genome. The genomes of two model legumes are currently being sequenced, *L. japonicus* by the Kazusa DNA Research Institute (<http://www.kazusa.or.jp/lotus/>) and *M. truncatula* by the *Medicago truncatula* Consortium (<http://www.medicago.org/genome/>). These model legumes have genomes of about 420 to 470 Mbp (Young et al. 2003), almost ten times smaller than that of the *A. hypogaea* genome (3,479 Mbp, <http://www.rbgekew.org.uk/cval/database1.html>). Thus, these conserved gene-based markers will greatly assist researchers to quickly identify orthologous genic markers in peanut. These will significantly speed up the identification of candidate genes for MAS and the positional cloning of genes for the development of transgenic varieties.

3.4.6

BAC Libraries and New Generation Markers

BAC Libraries

Bacterial artificial chromosome (BAC) libraries, providing whole-genome coverage in segments of about 100 kb, have become central to a wide range of goals in biology and genomics. Recently, the first large-insert DNA library for *A. hypogaea* was constructed (B. Yuksel and A.H. Paterson, manuscript in preparation). The library contains 182,784 clones; only 5,484 (3%) of them had no inserts; and average insert size is 104 kb. About 1,208 (0.66% of) clones appear to correspond to the 45S ribosomal DNA, and only 9 clones hybridize to chloroplast probes. The depth of coverage is estimated to be 6.5 times, allowing the isolation of virtually any single-copy locus. The identification of multiple loci by most probes in polyploids complicates anchoring of physical and genetic maps. The research group at the University of Georgia, Athens, GA, USA, explored the practicality of a hybridization-

based approach for determination of map locations of BAC clones in peanut by analyzing 94 clones detected by seven different overgos. The banding patterns on Southern blots were good predictors of contig compositions. This BAC library has great potential in terms of advancing the future research about the peanut genome.

Expressed Sequenced Tags (ESTs)

Hundreds of thousands of ESTs are available for soybean (Shoemaker et al. 2002; Tian et al. 2004), *M. truncatula* (Fedorova et al. 2002; Journet et al. 2002; <http://www.medicago.org>), and *Phaseolus* (Hernandez et al. 2004). In contrast, there are only 1,825 ESTs available for peanut derived from two cDNA libraries constructed using mRNA from immature pods of a drought-tolerant line (A13) and from leaves of tomato spotted wilt virus (TSWV) and leaf-spot-resistant line (C33-24): NCBI GeneBank accession numbers CD037499 to CD038843. These have been successfully used to develop 44 EST-derived SSR markers of which over 20% were polymorphic among 24 cultivated peanut accessions (Luo et al. 2003). This group has also arrayed about 400 unigenes of adversity resistance on glass slides. This macroarray is being used with mRNA probes from different lines that have been exposed to various profiles of drought stress or fungal infection to identify genes related to biotic or abiotic stresses. Another group at the University of Florida, Gainesville, USA, has constructed leaf, seed, and peg/pod cDNA libraries from developmentally pooled tissues of "SunOleic 97R" (peanut cultivar with high O/L ratio). These libraries are currently being sequenced to develop gene expression profiles that will lead to greater understanding of peanut's responses to various abiotic and biotic stresses. It is hoped that this will provide the necessary knowledge and tools to alter peanut to achieve maximum performance under given growth conditions (Chengalayan and Gallo-Meagher 2003). Aflatoxin is a serious quality problem in peanut. Drought and high-temperature stresses are conducive to *Aspergillus flavus* infection and aflatoxin contamination. Differential display reverse transcription PCR (DD-RT-PCR) (Liang and Pardee 1992) and EST/microarray are now used to locate multiple genes that enable plants to withstand biotic and abiotic stresses. Using DD-RT-PCR, Guo et al. (2003) revealed that some cDNA fragments are up- or down-regulated by induced drought stress and identified a novel *PLD* gene

that encodes a putative phospholipase D, a primary enzyme responsible for the drought-induced degradation of membrane phospholipids in plants. They studied the *PLD* gene expression under drought stress in the greenhouse using two peanut lines, Tifton 8 (drought tolerant) and Georgia Green (drought sensitive). Northern analyses showed that the *PLD* gene expression is induced sooner by drought stress in Georgia Green than in Tifton 8. After the *PLD* gene in peanut is characterized, the researchers plan to attempt gene silencing using genetic transformation to suppress *PLD* gene expression and induce drought tolerance.

An *A. flavus* ESTs program at USDA/ARS Southern Regional Research Center in New Orleans, LA (Yu et al. 2002) and USDA-ARS Labs at Tifton, GA has resulted in about 8000 expressed unique genes that will help to identify genes that could be used to inhibit fungal growth or aflatoxin formation by the fungi. Finally, a group at the University of Agricultural Sciences, Bangalore, India, has developed subtractive libraries for water use efficiency. ESTs from this project are being sequenced, and the most promising candidate gene markers will be mapped using an RIL population specifically designed for this purpose (Udaya Kumar et al. unpubl. data).

Transcriptional Profiling

Jain et al. (2001) used an RT-PCR-based procedure (differential display) to identify cDNA corresponding to transcripts affected by water stress in peanut and identified several mRNA transcripts that are up-regulated or down-regulated following water stress. With 21 primer combinations, they observed 1235 and 950 differential-display products in irrigated and drought-stressed samples, respectively. Forty-three peanut transcripts responsive to drought (PTRDs) were significantly altered due to water stress. Slot blot analysis of 16 PTRDs revealed that 12 were completely suppressed by prolonged drought while 2 were down-regulated, and 2 were up-regulated under drought-stress conditions. RNA dot-blot analysis of the 12 completely suppressed transcripts revealed that PTRD-1, PTRD-10, and PTRD-16 were expressed for a longer period in the tolerant line compared to the susceptible line. All these sequences may be useful candidate gene markers for mapping components of drought tolerance in peanut.

Variation in fatty acid profile is the major determinant of oil quality in peanut. Oils high in monounsaturated (oleic) and low in polyunsaturated fatty acids (linoleic and linolenic) are commercially and nutritionally desirable. Polyunsaturated fatty acyl residues are susceptible to oxidation, the products of which cause unpleasant odors and tastes commonly associated with rancidity. These oxidized products have potential atherogenic effects, while oils high in monosaturates have been reported to be effective in lowering cholesterol levels (St Angelo and Ory 1973; Broun et al. 1999). Mapping or isolation of genes associated with increased oleic acid accumulation would provide opportunities to alter fatty acid composition in peanut by MAS as has been achieved in soybean (Kinney and Knowlton 1998) and rapeseed (Friedt and Luhs 1999; Tanhuanpaa and Vilkki 1999). Jung et al. (2000a) isolated two cDNA sequences coding for microsomal oleoyl-PC desaturases (*ahFAD2A* and *ahFAD2B*) from the developing peanut seed with a normal oleate phenotype; these desaturases are nonallelic but homeologous genes originating from two different diploid species. The gene *ahFAD2A* is expressed in both normal and high oleate peanut seeds, but the *ahFAD2B* transcript is severely reduced in the high oleate peanut, suggesting that the reduction in *ahFAD2B* transcript level in the developing seeds is correlated with a high oleate trait. Further studies revealed that a mutation in *ahFAD2A* and a significant reduction in levels of the *ahFAD2B* transcript together cause the high oleate phenotype in peanut, and that of expressed gene encoding a functional enzyme appears to be sufficient for the normal oleate phenotype (Jung et al. 2000b).

Single Sequence Polymorphism (SNPs)

SNPs are the most elemental difference between genotypes, a difference in DNA sequence; therefore they are the most direct means of DNA fingerprinting that can ever exist. SNPs have replaced SSRs as the preferred marker in mammalian genomics. A wide range of emerging, high-efficiency techniques for finding SNPs, even when polymorphism is rare (such as in peanut), sets the stage for use of genomic tools on a scale not previously possible. SNPs provide enabling biotechnologies in the form of low-cost molecular markers and genetic fingerprinting tools suitable not only for plant variety protection but broadly applicable to the implementation of environmentally friendly

genetic solutions to challenges that increase the economic and environmental costs of peanut production.

SNP discovery in polyploids such as peanut poses a problem not faced in diploids, i.e., that most PCR amplification products are likely to be mixtures of sequences from two or more divergent loci. This precludes many otherwise attractive SNP discovery strategies based on direct resequencing of PCR products. It remains to be determined exactly the degree to which this will be a problem in peanut as there is very little comparative sequence data for both cultivated peanut and its diploid progenitors. Further, the antiquity of polyploid formation in peanut will also bear on this problem – if polyploid formation were ancient, there might be an appreciable degree of “diploidization” or loss of some duplicated gene copies, suggesting that reasonable populations of truly single-copy loci might be found. However, polyploid formation is thought to be relatively recent in view of the generally low polymorphism rate. Further investigation of the structure and evolutionary history of the peanut genome will be needed to evaluate various SNP discovery strategies and implement optimal strategies across the genome and the gene pool.

A few SNPs for particular high-priority genes have already been discovered. Lopez et al. (2000) used peanut lines with a low (T-90) or high (F435) oleic (O) to linoleic (L) fatty acid ratio to isolate and characterize the Δ^{12} -fatty acid desaturase (FAD) gene. The Δ^{12} -FAD contains a putative intron, the coding region at the 3 end, and an open reading frame (ORF) of 1,140 bp encoding 379 amino acids. A comparison of coding sequences from the high and low oleic acid genotypes revealed several SNPs: one SNP in the flanking region at 229 bp upstream of the start codon and a cluster of four SNPs in the coding region. Two polymorphisms appear to be associated with the high O/L trait. The first is an “A” insertion 442 bp after the start codon that shifts the amino acid reading frame, probably resulting in a truncated, inactive protein and the loss of one of three histidine boxes believed to be involved in metal ion complexation required for the reduction of oxygen and another polymorphism at 448 bp from the start codon that results in an amino acid change. Several independently derived backcross lines with high O/L ratio had either the “A” insertion or the amino acid substitution. This association of the molecular polymorphisms with the low and high oleate trait in peanut should allow peanut breeders to develop an effective

tive and low-cost molecular assay for the high O/L trait.

Resistant Gene Analog (RGA)

Plants have distinct mechanisms for defending themselves against diseases. One of these involves the specific recognition of, and response to, pathogens. Many of the genes that control this type of resistance encode proteins with an NBS (nucleotide-binding site) domain (Meyers et al. 1999). The only function so far associated with the NBS in plants is disease resistance.

Amino acid motifs within the NBS can be used to design degenerate PCR primers that amplify diverse NBS encoding regions from plant genomic or total cDNA. These NBS encoding regions, isolated by cloning and converted into genetic markers, have, in some studies, been shown to be genetically linked to known R-genes, or indeed to be fragments of the known R-genes themselves (Kanazin et al. 1996; Aarts et al. 1998; Collins et al. 1998, 1999, 2001; Shen et al. 1998; Hayes and Saghai-Marouf 2000; Donald et al. 2002). NBS encoding regions are therefore ideal candidate gene markers for disease resistances in peanut. There is a very large number of NBS encoding regions in plant genomes (about 150 in *Arabidopsis* and many more in larger genomes) potentially creating a high level of redundancy for this process. However, since many resistance genes occur in clusters it may not be necessary to specifically detect the correct candidate gene but instead effective MAS systems may be derived from any resistance gene analog in the correct genomic region. Thus, Bertoli et al. (2003) have used degenerate primers to isolate 78 complete NBS encoding regions from genomic DNA of a number of *Arachis* species.

EMBRAPA and UCB have been working to convert these NBS sequences to molecular markers for the diploid AA genome mapping population (see Sect. 4.3 for further details). Southern blots using NBS-based probes show high polymorphism, in many cases cosegregation of homologs, and often differences in the numbers of homologs between the mapping parents. In our opinion it certainly is worthwhile to place the major resistance gene clusters on the *Arachis* genetic map. Incorporated within a framework of transferable PCR-based markers (SSRs), these markers should serve as

a good resource for the peanut research and breeding community.

3.5

Successes and Limitations of Conventional Breeding in Peanut

Progress in conventional peanut breeding has recently been reviewed elsewhere (Dwivedi et al. 2003a). Peanut-breeding programs, in developed and developing countries, have made significant progress toward developing cultivars with crop durations ranging from 90 to 150 d and pod yield potentials from 3.0 t ha⁻¹ to 9.0 t ha⁻¹. However, farmers in most countries do not come close to realizing these types of yields (i.e., world average yield of 1.35 t ha⁻¹). The highest average national yields are 2.6 t ha⁻¹ in China and 3.5 t ha⁻¹ in the USA (FAO 2003), although even higher yields have been reported in isolated farmers' fields in China and Zimbabwe (Smartt 1978; Yanhao and Caibin 1990).

Resistance to rust, bacterial wilt, and groundnut rosette virus (GRV) has been successfully incorporated into improved genetic background. Bacterial-wilt-resistant cultivars in Southeast Asia and the Far East and GRV-resistant cultivars in sub-Saharan Africa are now grown on large areas. A few cultivars with moderate resistance to rust and leaf spots have also been developed; however, these have not become popular among farming communities in the semiarid tropics because of their relatively long duration, low shelling out-turn, and inferior pod/seed characteristics compared to preferred cultivars. More recently, several peanut-breeding programs have been successful in diluting this undesirable linkage, facilitating the development of breeding lines with a shorter duration and moderate resistance to rust and/or LLS plus excellent pod/seed characteristics (Upadhyaya et al. 2002b).

In contrast, peanut breeders have not been successful in developing cultivars that show complete resistance to *Aspergillus flavus* in order to eliminate aflatoxin contamination. Several germplasm accessions and breeding lines are available that offer various components of genetic resistance (such as resistance to pod infection, to seed invasion, and to aflatoxin production). But again, when these traits are introgressed into breeding programs, the genetic resistance has not

improved over what is already available in germplasm lines.

Interspecific crossing and selection has resulted in the release of two nematode resistant varieties, Coan and NemaTAM, in the USA (Simpson and Starr 2001; Simpson et al. 2003). Conventional breeding has had some success in selecting for drought tolerance in peanut. However, trait-based (specific leaf area, water-use efficiency, amount of water transpired, transpiration efficiency, and harvest index) selection is likely to be a more rewarding strategy to substantially enhance drought tolerance. ICRISAT has developed breeding lines originating from trait-based selection that are being compared with breeding lines originating from conventional selection for their response to drought and yield potential (Nigam et al. 2003a).

Peanut oil quality is determined by the ratio of oleic (O) fatty acid/linoleic (L) fatty acid: a higher ratio results in a better storage quality of the oil and longer shelf life of peanut products. With the availability of peanut germplasm with an exceptionally high O/L fatty acid ratio (Norden et al. 1987), US peanut breeders have been successful in transferring this trait into improved genetic backgrounds, and several newly developed cultivars with improved oil chemistry are now commercially grown in the USA.

3.6 Biotechnological Applications to Genetic Enhancement in Peanut

3.6.1 Marker/Trait Associations

Unlike with other oilseed crops such as soybean, oilseed rape, and sunflower, the genomics and molecular breeding of peanut is still in its infancy. Although peanut is a complex polyploid (like oilseed rape), the primary reason for slow progress is the lack of detectable molecular variation in cultivated peanut. This problem has been somewhat resolved by the large-scale development of SSR markers. A few economically important traits have now been mapped in peanut. Resistance to root-knot nematode has been mapped using RFLP and/or RAPD assays in an interspecific cross *A. hypogaea* Florunner × wild *Arachis* species. Two dominant genes confer resistance to root-knot nematode: *Maer* restricts egg number and

Mag gall formation. RAPD and RFLP markers closely linked to the resistance loci have been identified (Table 6). The RFLP loci R2430E and R2545E are easy to score and sufficiently close to the resistance allele for an acceptable selective power. Similarly, a RAPD marker, Z3/265, linked at 10 cM and 14 cM from *Mag* and *Mae*, respectively, has been converted into a SCAR marker and RFLP probe that confirmed linkage with nematode resistance. Association of an RFLP probe R2430E linked to a locus for resistance to root-knot nematode race 1 in four breeding populations has further validated these markers. US peanut breeders now routinely use these markers to select for nematode resistance. RAPD markers associated with resistance to southern corn rootworm, ELS, and cylindrocladium black rot have also been reported (Table 6). Aphid is a vector of GRV that causes substantial yield losses in peanut production in sub-Saharan Africa, and identification of markers linked with aphid resistance should help peanut breeders select for combined resistance to vector and the GRV. A single recessive gene that confers resistance to aphid has been mapped on LG 1, 3.9 cM from a marker originating from the susceptible parent explaining 76% of the phenotypic variation for aphid resistance (Herselman et al. 2004).

Resistance to a number of diseases (ELS and LLS, rust, and bacterial wilt) is being mapped using SSR and AFLP markers at ICRISAT. Preliminary results have identified a number of markers closely linked to QTL for each resistance trait (Mace et al. unpubl. data).

Urgent needs include the development of large numbers of user-friendly genetic mapping tools; sequencing of substantial populations of expressed sequences from diverse tissues, genotypes, and stress profiles; assembly of a genetically anchored physical map and its alignment to the emerging model legume genomes such as *Lotus* and *Medicago*; and sampling the gene-rich regions to quantify the additional information that may be gained by further sequencing of the peanut genome (Paterson et al. 2004).

3.6.2 Unlocking the Genetic Variation from Wild Genetic Resources

Although there is high morphological diversity among varieties and landraces of *A. hypogaea*, molecular genetic diversity and variability for some important traits of agronomic interest are low.

Table 6. Summary of marker-trait relationships reported in mapping populations derived from interspecific crosses in peanut

Trait	Summary of DNA markers linked with beneficial traits	Reference
Root-knot nematode (<i>Meloidogyne arenaria</i> (Neal) Chitwood)	<i>Arachis hypogaea</i> × Wild <i>Arachis</i> species crosses BSA identified RAPD markers, RKN410, RKN440, and RKN229, linked with nematode resistance in BC ₄ F ₂ population of the cross Florunner × TxAG7 and further validated by screening 21 segregating BC ₄ F ₂ and 63 BC ₅ F ₂ single plants. Recombination fraction between RKN410 and resistance and between RKN440 and resistance was 5.4 cM and 5.8 cM, respectively. These two markers identified a resistance gene derived from either <i>A. cardenasii</i> or <i>A. digoi</i> and were closely linked to each other. Marker RKN229, that inherited from <i>A. cardenasii</i> or <i>A. digoi</i> , was 9 cM away from resistance locus.	Burow et al. 1996
	Two dominant genes conditioning resistance to the root-knot nematode reported in segregating F ₂ populations involving a root-knot nematode introgression line GA 6 (<i>A. hypogaea</i> × <i>A. cardenasii</i>) and a highly susceptible recurrent parent PI 261942. The gene <i>Mae</i> restricts egg number and <i>Mag</i> gall formation. A RAPD marker Z3/265 was linked at 10 cM and 14 cM from <i>Mag</i> and <i>Mae</i> , respectively. They cloned this marker to make SCAR and RFLP probes, and these markers confirmed the linkages with nematode resistance.	Garcia et al. 1996
	An RFLP probe R2430E linked to a locus for resistance to <i>Meloidogyne arenaria</i> race 1 in four breeding populations and three peanut lines, demonstrating that RFLP probe R2430E linked to nematode resistance provide a useful selection method for identifying resistance to the peanut root-knot nematode.	Seib et al. 2003
	Three RFLP loci (R2430E, R2545E, and S1137E) linked with resistance to nematode at distances of 4.2 to 11.0 cM in BC ₂ F _{2:4} population of the cross Florunner × TxAG7. R2430E and R2545E are easy to score and sufficiently close to the resistance allele that can be used with a high level of confidence to select resistant progenies based on marker information.	Choi et al. 1999; Church et al. 2000
Early leaf spot and southern corn rootworm	Evaluated six polymorphic RAPD markers (AD 1, AI 11, AI 19, AJ 19, AK 20, and AN 15) for components of resistance to early leaf spot (ELS) and southern corn rootworm resistance in F ₂ population involving <i>A. hypogaea</i> cultivar (NC 7) and ELS resistant tetraploid interspecific derivative NC GP WS 1, and established association between RAPD markers and sporulation, lesion diameter, and defoliation and for southern corn rootworm resistance.	Stalker and Mozingo 2001

Table 6. (continued)

Trait	Summary of DNA markers linked with beneficial traits	Reference
<i>Arachis hypogaea</i> × <i>Arachis hypogaea</i> crosses		
Early leaf spot and cylindrocladium black rot	Cylindrocladium black rot resistance was associated with RAPD marker AM 1101 and ELS sporulation was associated with AM 1102 in F ₂ population of the cross NC 7 × PI 109839.	Stalker and Mozingo 2001
Aphid	Aphid resistance in ICG 12991 was mapped on linkage group 1, 3.9 cM from a AFLP marker originating from the susceptible parent, that explained 76.1% of the phenotypic variation for aphid resistance.	Herselman et al. 2004

This is because of an extreme genetic bottleneck at the origin of this species. Peanut evolved through the hybridization of two wild diploid species followed by spontaneous duplication of chromosomes. The resultant allotetraploid (or amphidiploid) plant would have captured good hybrid vigour but been reproductively isolated from its wild relatives. There are also good reasons to believe that the lack of allelic diversity in *A. hypogaea* has also led to genetic restrictions to increasing productivity.

The best sources of disease resistance genes are found in wild species (Dwivedi et al. 2003a). *A. monticola* is the only wild relative that is sexually compatible with cultivated *A. hypogaea*. A few cultivars including Spancross and Tamnut 74 have been developed that include *A. monticola* within their ancestry (Isleib et al. 2001). However, the bulk of agronomically useful genetic diversity lies in the diploid species. There are three main pathways that have been proposed for the incorporation of this diversity into breeding programs (Simpson 2001):

- (1) A diploid wild species is crossed with *A. hypogaea* to generate a sterile triploid hybrid. This hybrid is treated with colchicine to double the chromosomes and produce a hexaploid plant with 60 chromosomes, which is crossed and backcrossed with *A. hypogaea* until the progeny regains the normal chromosome number of 40.
- (2) Two wild species, one with genome type AA and the other BB, are treated with colchicine to create tetraploids. These are then crossed to give a plant with a genome type AABB that is then crossed and backcrossed with *A. hypogaea* to regain the

cultivated agronomic background while selecting for the exotic trait of interest.

- (3) Two wild diploid plants are crossed; the primary hybrid is treated with colchicine to double the chromosomes and produce a synthetic amphidiploid (allotetraploid). This amphidiploid is then crossed and backcrossed with *A. hypogaea*. This pathway is likely to be most successful when species with AA and BB genomes are used to make this primary cross, as synthetics with other genomes may not be readily cross fertile with cultivated peanut.

The first pathway has been successfully used for the development of new varieties (reviewed by Dwivedi et al. 2003a and Holbrook and Stalker 2003). The second pathway is reported to have had limited success because of sterility problems. The third (resynthesis) pathway essentially attempts to artificially recreate events similar to those that gave rise to the evolutionary speciation of *A. hypogaea*. A variant of this pathway in a cross has led to the development of cultivars that incorporate wild resistance genes (Simpson and Starr 2001; Simpson et al. 2003). The crossing used a hybrid between two AA-genome species (*A. cardenasii* and *A. diogoi*) as the A donor crossed with *A. batizocoi* as the B donor. However, it is now known that *A. batizocoi* has a very different genome to the B genome of *A. hypogaea*. For instance, the chromosomes of *A. batizocoi* have centromeric heterochromatic bands absent in the B genome of *A. hypogaea*. Nevertheless, these contrasting differentiations did not lead to the sterility problems that might have been expected.

Nowadays our knowledge of the affinities of the genomes of species within the taxonomical section *Arachis* are much better defined. In particular, the wild species with genomes most similar to the ancestral genomes of *A. hypogaea* have been identified: *A. duranensis* as the contributor of the A genome and *A. ipaensis* as the contributor of the B genome (Seijo et al. 2004). With this in mind EMBRAPA scientists, in collaboration with Charles Simpson of Texas A&M University, USA, has recently undertaken work to "resynthesize" *A. hypogaea* using the ancestral and related species (A. Fávoro et al. unpubl. data). This approach of resynthesis is attractive because it may minimize both sterility barriers and suppression of recombination, both major barriers in the utilization of wild species in breeding.

The resynthesis of allopolyploid crops has been successfully used for introgressing exotic traits in both oilseed rape (Akbar 1989; Chen and Heneen 1989; Lu et al. 2001) and wheat (Fernandes et al. 2000). So far, five synthetic *Arachis* amphidiploids have been generated: [*A. hypogaea* × (*A. ipaensis* × *A. duranensis*)]^c, *A. hypogaea* × (*A. hoehnei* × *A. cardenasii*)^c, *A. hypogaea* × (*A. aff. magna* × *A. villosa*)^c, (*A. aff. magna* × *A. aff. diogoi*)^c, and (*A. hoehnei* × *A. helodes*)^c. Fertile hybrids from crosses between three of these with *A. hypogaea* × (*A. hoehnei* KG30006 × *A. cardenasii* GKP10017)^c, *A. hypogaea* × (*A. aff. magna* V6389 × *A. villosa* V12812)^c, and *A. hypogaea* × (*A. ipaensis* KG30076 × *A. duranensis* V14167)^c have been obtained. The ^c in the description of the amphidiploid crosses indicates that the plants have been treated with colchicine and have chromosome number 2n = 40.

The accessions used for these crosses were chosen based on the results of bioassays with late leaf spot (LLS) [*Phaeoisariopsis personata* (Berk. and Curtis) Deighton] and rust (*Puccinia arachidis* Speg) isolates from Brazil. It was noteworthy that all of the 97 wild accessions tested had higher levels of resistance than the 10 control cultivars of *A. hypogaea*, and that there was great heterogeneity within species as regards disease resistance. Therefore, it is invalid to regard a particular species as being uniformly resistant against any of the fungi tested. These synthetic amphidiploids incorporate new disease resistance genes from both the A and B genomes. The usefulness of resistances of these wild sources has been further confirmed in greenhouse screens using a severe combined challenge with LLS and rust. All *A. hypogaea* cultivars were severely affected, many losing

almost all of their photosynthetic leaf area, while all synthetic amphidiploids and their F₁ hybrids with *A. hypogaea* showed high levels of resistance (A. Fávoro et al. unpubl. data).

In order to efficiently use the synthetic amphidiploids in prebreeding, it will be necessary to apply foreground and background marker-assisted introgression and backcross breeding. Genetic maps, constructed with markers that have good transferability across species and that are reasonably easy to use (SSRs would seem to be the best choice), will facilitate the effective introgression of target traits while simultaneously efficiently eliminating the remainder of the wild species genome. This approach will be heavily dependent on the microsatellite-based genetic maps that have already been developed for the A genome and that are ongoing for the B genome. These diploid maps will be validated in a tetraploid population derived from a cross of an amphidiploid with *A. hypogaea*.

The combination of SSR-based genetic maps of diploid species and synthetic amphidiploids incorporating various exotic genomes are first steps toward a new molecular-enhanced paradigm in peanut breeding that will unlock the value of wild *Arachis* germplasm that has been hitherto largely beyond the reach of most breeders. New peanut varieties incorporating wild *Arachis* genes will have improved resistance to biotic stresses and tolerance to abiotic stress together with allelic combinations for enhanced yield potential and increased quality profiles that would never have been possible through conventional approaches.

3.6.3

Transgenics

An efficient tissue culture and transformation system to introduce foreign DNA into peanut has been reported (Sharma and Anjaiah 2000), and transgenic peanuts carrying genes for resistance to TSWV, lesser cornstalk borer, and sclerotinia blight in the USA; a gene carrying resistance to peanut stripe virus in China; and genes carrying *IPCV_{cp}* or *IPCV_{replicase}* for resistance to peanut clump virus, *GRAV_{cp}*, for resistance to groundnut rosette assistor virus (GRAV), rice *chitinase* for resistance to fungal diseases, and drought responsive elements of *Arabidopsis* (rd29A:DREB1A) for improving drought tolerance have been produced at ICRISAT (Table 7). These transgenics are in var-

Table 7. List of transgenics carrying genes for resistance to peanut clump virus, groundnut rosette assistor virus, tomato spotted wilt virus, peanut stripe virus, fungal and soil born diseases, drought and allergens in peanut

Trait	Gene and host cultivar	Current status of transgenics	Reference
Peanut clump virus (PCV)	<i>IPCV_{cp}</i> or <i>IPCV_{replicase}</i> gene	2-year field trials of transgenic peanuts having coat protein-mediated resistance (<i>IPCV cp12</i> , <i>IPCV cp12</i> , and <i>IPCV cp51</i>) or replicase-mediated resistance (<i>IPCV rep3</i>) have consistently shown resistance to PCV.	ICRISAT 2004
Groundnut rosette assistor virus (GRAV)	<i>GRAV_{cp}</i> gene	Over 50 T ₂ -T ₃ generation transgenic lines containing <i>GRAV_{cp}</i> gene characterized for gene integration and expression using RT-PCR and Southern hybridization.	ICRISAT 2004
Tomato Spotted Wilt Virus (TSWV)	Nucleocapsid protein gene	The transgenic peanut showed lower TSWV incidence in comparison to nontransgenic control, both in the field and in controlled environment conditions.	Yang et al. 2004
Peanut Stripe virus (PStV)	Coat protein gene	Transgenic plant carrying copies of viral coat protein gene exhibited high levels of resistance to PStV.	Dietzgen et al. 2004
Fungal diseases (rust and leaf spots)	Rice chitinase gene	Thirty-six transgenic plants were evaluated for resistance to rust using a detached leaf technique and at 25 d after inoculation, 7 and 20 transgenic plants showed over 85% and 50% reduction in rust pustules, respectively.	ICRISAT 2004
Sclerotinia blight (<i>Sclerotinia minor</i>)	Antifungal gene	A 3-year study of evaluation of several transgenic lines containing antifungal gene under high disease pressure revealed an average reduction of 32% <i>S. minor</i> infection compared to susceptible control Okrun and were comparable for shelling percentage and 100-seed weight to that of nontransgenic control Okrun. Two lines consistently averaged <i>S. minor</i> incidence similar to resistant control Southwest Runner.	Chenault and Melouk 2003
	Oxalate oxidase gene	Transgenic peanuts containing oxalate oxidase gene, evaluated in greenhouse, expressed higher levels of oxalate oxidase activity than untransformed controls. 79% of Perry, 78% of Wilson, and 35% of NC7 transgenics showed significantly elevated expression, and few selected lines showed 3 to 4 times as much activity as untransformed controls.	Livingstone et al. 2003
Drought	rd29A:DREB1A	T ₂ transgenic lines revealed positive gene integration and expression. DDRT-PCR test performed on these lines, subjected to various abiotic stresses (salinity, high and low temperature) under in vitro conditions, revealed the up and down regulation of several mRNAs besides identifying some new mRNA clones.	ICRISAT 2004
Allergens	<i>p</i> DK2 construct and a plasmid <i>p</i> CB13 containing hygromycin marker	Stable transgenic peanut with knock down expression of <i>Ara h2</i> gene produced and Northern hybridization revealed that <i>Ara h2</i> gene is expressed only in peanut seeds and not in vegetative tissues.	Konan et al. 2002

ious stages of characterization under containment glasshouse and/or controlled field evaluations. In addition, Peggy Ozias-Akins's group in Tifton/Georgia, USA, has adopted a three-tier approach to develop transgenic peanut by incorporating resistance to insect damage using a *Bt* gene, resistance to fungal growth using the tomato anionic peroxidase gene (*tap1*), or antifungal peptide D4E1, and inhibition of aflatoxin biosynthetic pathway using the lipoxygenase gene *lox1* (Ozias-Akins et al. 2002). Transgenics clearly offers tremendous potential for introgressing genes not available in the *Arachis* genus or that have pleiotropic effects on yield potential or quality profiles.

Once favorable genes have been introduced into a cultivated peanut genotype, they can quickly be introgressed in a wide range of locally adapted and preferred backgrounds through marker-assisted breeding.

3.7

Conclusions and Future Outlook

The genus *Arachis* probably arose during the tertiary period, and the genus now contains genetically diverse accessions and species. Most of this variation is not directly available to plant breeders because genes of interest are within genomes that are too diverged from the genome of *A. hypogaea* for sexual compatibility and too large and uncharacterized to allow positional cloning. The genomes of accessions within the taxonomical section *Arachis* are, however, available through the routes detailed in Sect. 6.2. To date, however, there are very few examples of released cultivars that contain genes from wild species.

The events that gave rise to *A. hypogaea* imposed a severe genetic bottleneck at the origin of the crop, and the genetic diversity in cultivated germplasm today results from only some 4,000 years of mutation and selection. In addition, a second genetic bottleneck has been imposed by modern breeding programs, which so far have only used a tiny fraction of the variation within *A. hypogaea*. Therefore, commercial cultivars grown today have a very narrow genetic base, and the allelic combinations available from working with elite germplasm are limited. Therefore, there is an urgent need to broaden the genetic base of cultivated peanut germplasm.

Genetic resources of peanut currently available in germplasm banks consist of ca. 15,000 cultivated and 400 wild *Arachis* species accessions maintained at ICRISAT, USDA, and CENARGEN. These genetic resources harbor genes for resistance/tolerance to biotic and abiotic stresses in addition to showing variability for a range of morphophysiological, reproductive, and seed quality traits. There are also two well-defined core and minicore collections representing the majority of variation present in the cultivated peanut germplasm. These are good resources to analyze genetic relationships and detect allelic variation linked with beneficial traits through association mapping, and they provide an effective entry point to the entire collection. Peanut genomics has progressed rapidly during the past decade such that the peanut genomic resources now include availability of a large number of RAPD, AFLP, RFLP, and SSR markers with EST and SNP markers just beginning to emerge. These markers are being used in genetic diversity and marker-trait associations and for the development of genetic linkage maps. An RFLP-based map of tetraploid *Arachis*, derived from an interspecific backcross population, is already available to peanut researchers. However, it has limited value to peanut breeders as the RFLP loci placed on this map are unlikely to detect polymorphic alleles in intraspecific cultivated *A. hypogaea* crosses. There is an urgent need to develop more PCR-based genetic linkage maps as only a few sparsely spaced AFLP- and SSR-based genetic maps have been reported for intraspecific *A. hypogaea* crosses. Similarly, there is need to saturate the preexisting maps with more PCR-based markers. Considerable effort has been directed toward generating new SSR markers. EST and SNP markers are already available for oil quality and drought tolerance, but large-scale development of EST, RGA, and SNP markers will now be of substantial importance. Efforts are also being directed toward developing an SSR-based linkage map of the A and B diploid genomes that should enable mapping of exotic and/or complex traits (especially polygenic ones) that would have been difficult or impossible to deal with in a conventional tetraploid background. Peanut is also being included in the development of a consensus legume genetic linkage map using legume family anchor markers. Eight hundred sixty-seven evolutionary conserved sequences (ECSs) that are likely to be well conserved within the legumes have been identified, and these are being used for marker development. Comparison of the map positions of these markers in different legumes should

allow the development of a single genetic framework map for legumes that in turn should assist peanut researchers to use genomic information from the model plants and facilitate the generation of genic markers, the identification of candidate genes, and positional cloning in *Arachis*.

Transgenic peanut carrying genes for resistance to several fungal and virus diseases and for some insect pests, which are in various stages of evaluation, will be available to peanut researchers for introgression into their target peanut cultivars. It is proposed to adopt an approach that combines transgenic techniques, MAS, and conventional breeding to provide intrinsic, low-cost, and environmentally benign solutions to the many challenges that increase the cost and risk of peanut production and cause peanut to fall short of consumer needs and desires.

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