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Wild Crop Relatives: Genomic and Breeding Resources

Legume Crops and Forages

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Chapter 1
Arachis

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1.1 Introduction

Crop wild relatives (CWR) are wild plant taxa that have an indirect use derived from their relatively close genetic relationships to crops (Maxted et al. 2006). An understanding of the taxonomic and evolutionary relationships between cultigens and their wild relatives is prerequisite for the exploitation of wild relatives in crop improvement programs (Hawkes 1977). In the past, several reviews on the use of wild relatives for crop improvement have been published, which demonstrated greatest benefit towards improving the levels of resistance to pests and diseases in several crops including groundnut (Harlan 1976; Stalker 1980; Goodman et al. 1987; Lenne and Wood 1991; Hoisington et al. 1999; Dwivedi et al. 2003). Hajjar and Hodgkin (2007) documented information on the presence of genes from CWR in released cultivars of CGIAR mandate crops, demonstrating that there has been steady increase in the rate of release of cultivars containing genes from CWR. More recently, it has also been demonstrated that CWR have contributed alleles associated with increased fruit/grain yield and improved seed quality, predominantly in tomato and rice, and resistance to drought and salinity in wheat [reviewed in Dwivedi et al. (2008)].

Groundnut (*Arachis hypogaea* L.) originated in South America and is widely grown (113 countries) throughout tropical, subtropical and warm temperate regions (40°N to 40°S). Worldwide, groundnut is next in importance after soybean and rapeseed, with an annual production of 38.2 million tons and average productivity of 1.5 ton ha^{-1} (FAO 2008). The seeds are rich in oil and protein and are eaten in a variety of forms. About two-thirds of global production is crushed for extracting vegetable oil. The remaining one-third is used in the form of edible product and as seed. The cake obtained after oil extraction is used as protein-rich meal for livestock or for making other food products. The haulms are an important source of good quality animal fodder. Some of the perennial wild species, such as *A. glabrata* from the section Rhizomatosae, have been used to develop several commercial tropical forage cultivars, including the Florigraze and Arbrook in the USA that are used as an alternative to alfalfa because of their high levels of proteins and resistance to pest and diseases (Prine et al. 1981, 1986; French et al. 1994). Likewise, in Australia, *A. glabrata* is valued as high-quality forage having the ability to spread through swards of aggressive summer-growing grass species (Bowman et al. 1998). In addition, groundnut helps to improve soil fertility through biological nitrogen fixation.

Rust, early leaf spot, and late leaf spot are the most common and widely distributed foliar diseases of groundnut worldwide, while leaf minor is common in South Asia; army worm (*Spodoptera*) and bacterial wilt in South-east Asia; groundnut rosette disease and termite in Africa; and nematode, corn earworm, lesser corn stock borer, and southern corn rootworm in North America. Some insects are also the vectors of important viral diseases – *Thrips palmi* for peanut bud necrosis virus, *Frankliniella occidentalis* and *F. fusca* for tomato-spotted wilt virus and *Aphis crassivora* for groundnut rosette virus. In addition to biotic stresses, the crop is also adversely affected by drought, salinity, low availability of phosphorus under acidic soils and nonavailability of iron in calcareous soils in many

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parts of the world. Aflatoxin contamination is the major problem adversely affecting the groundnut seed quality. All these factors either alone or in combination adversely affect the yield and/or quality worldwide, necessitating the identification and utilization of resistance sources to enhance and sustain groundnut production. With regard to several pests and diseases, the level of resistance required is either not present or available only at very low levels in cultivated groundnut, while very high levels of resistance to pests and diseases have been reported in many wild *Arachis* relatives [reviewed in Dwivedi et al. (2003)].

The cultivated groundnut, *A. hypogaea*, belongs to the section *Arachis*, which also contains its tetraploid progenitor *A. monticola* Krapov. and Rigoni (Favero et al. 2006), and 29 wild diploid species that are cross-compatible with *A. hypogaea*. *A. ipaënsis* and *A. duranensis* have been suggested as putative B- and A-genome donors, respectively, of the cultivated peanut (Kochert et al. 1996; Seijo et al. 2004; Favero et al. 2006). More recently, Seijo et al. (2007) used the double genomic in situ hybridization (GISH) technique on seven diploid species that harbored either the A- or B-genome, to provide further evidence that *A. duranensis* (A-genome) and *A. ipaënsis* (B-genome) are the best candidates for the genome donors of cultivated groundnut as both yielded the most intense and uniform hybridization pattern when tested against the corresponding chromosome subsets of *A. hypogaea*. Further, all the presently known subspecies and varieties of *A. hypogaea* have arisen from a unique allotetraploid plant population, or alternatively, from different tetraploid populations that originated from the same two diploid species.

Singh and Simpson (1994) have classified the genetic variability in the genus *Arachis* into four genepools: primary gene pool (landraces of *A. hypogaea* and its wild form *A. monticola*), secondary gene pool (diploid species from section *Arachis* that are cross-compatible with *A. hypogaea*), tertiary gene pool (species of section *Procumbentes* that are weekly cross-compatible with *A. hypogaea*) and the fourth gene pool (wild *Arachis* species classified into seven other sections). While interspecific crosses involving some species from secondary gene pool have been successful in groundnut, it is more difficult to cross species from tertiary and forth gene pool, for which, techniques such as in vitro culture of ovule and embryo is a must to produce viable hybrids (see Sect. 1.7).

This review is devoted to the use of wild *Arachis* for the improvement of *A. hypogaea* (cultivated groundnut) with the focus on conservation and regeneration of wild *Arachis* genetic resources; geographical distribution and the need to expedite collection of those species not present in genebanks before these are lost due to climate change or habitat disturbances in South America; differences in ploidy levels, genomes, and crossing relationships; descriptors used to characterize *Arachis* species; sources of resistance to biotic and abiotic stresses and for seed quality; barriers to interspecific hybridization; genomic resources developed to facilitate introgression of beneficial traits from wild *Arachis* to *A. hypogaea*; approaches to interspecific gene transfer and use of genetic markers to demonstrate the introgression of traits from wild *Arachis* species; the elite germplasm and cultivars developed using wild *Arachis* species; and new approaches to unlock the genetic variation from wild relatives using appropriate genetic and genomic resources.

1.2 Wild *Arachis* Species

1.2.1 Geographical Distribution

Arachis is exclusively a genus of South America and consists of nine sections that comprise 80 annual and perennial species (Krapovickas and Gregory 1994; Valls and Simpson 2005). It belongs to the family Leguminosae-Papilionoideae, tribe Aeschynomeneae and subtribe Stylosanthinae, and is restricted to Argentina, Bolivia, Paraguay and Uruguay. The *Arachis* section species occur in Brazil (mostly in the west central region) followed by Paraguay, Argentina and Uruguay. Wild *Arachis* species occur both in open and shaded areas, ranging from near to the equator to 34°S and from sea level to an attitude of almost 1,600 m. Because of the geocarpic nature of the fruit, species distribution generally follows major river valleys. Infrageneric groups may be closely associated with specific drainage basins, such as members of the section Triseminatae are found in the São Francisco,

while species of the section *Arachis* in the drainage basin of the river Paraguay and also in the Amazon drainage basin. Some overlap in distribution does occur for the sections Arachis, Erectoides, Rhizomatosae and Extranervosae (Gregory et al. 1973; Valls 1983; Valls et al. 1985). Species in the section Arachis are distributed in Argentina, Bolivia, Brazil, Uruguay and Paraguay, from the southern extreme of the genus along the river Uruguay to the eastern most extreme of the genus in Bolivia and Argentina and north-eastwards across the Brazilian Highlands. Section Heteranthae contains six species and is endemic to the north-eastern highlands of Brazil, while section *Trierectoides* contains two species, *A. guaranitica* and *A. tuberosa*, and is geographically restricted to a narrow distribution range in Brazil (one population of *A. guaranitica* is also reported from Paraguay). Species in section *Caulorhizae* including *A. pintoi* and *A. repens* are endemic to Brazil and centered in the eastern Brazilian highlands with scattered populations found towards the highlands of Mato Grosso do Sul. Section *Procumbentes* species are distributed where the borders of Paraguay, Bolivia and Brazil come together, near an area known as Pantanal while Erectoides section species are restricted largely in the Brazilian Province of Mato Grosso do Sul stretching southwards in Paraguay. Section *Extranervosae* species are also endemic to Brazil, inhabiting the Brazilian Highlands north and west of Mato Grosso do Sul, spreading across the Brazilian Plateau as far as 5°S. Section *Triseminatae* is endemic to the north-eastern Brazilian Highlands, while section *Rhizomatosae* species inhabit areas surrounding the Parana basin, and southwards through Paraguay, Argentina and into Uruguay, following the Rio Paraguay and meeting the Rio Uruguay (Ferguson et al. 2005).

1.2.2 *Ex Situ* Conservation of Wild *Arachis* Genetic Resources and Priority Areas for Future Collection

The major centers of conservation of wild *Arachis* species are in India, Brazil, USA, Argentina and Columbia, together holding ~2,800 accessions (Table 1.1). ICRISAT developed *Arachis* house, an open space fixed with a large cylindrical concrete structure (75 cm high, 90 cm in inner ring diameter, and of 5 cm ring thickness) with a ring-to-ring distance of 52.5 cm, for regenerating the seeds of wild *Arachis* species (Fig. 1.1). These rings are filled with about 0.5 m^3 pasteurized [3 cycles of 1 h each at 82°C and 34.5 × 10^3 Pa (5 psi)] soil mixture (soil, sand and FYM in 3:2:1 ratio). Five to six plants can be accommodated in one ring. After harvesting the pods at maturity, the remnant pods/seeds are visually collected and destroyed to avoid contamination with the next seed lot. The rings are kept fallow for 2–3 months and 2–3 irrigations are provided to allow remnant seeds, if any, to germinate, which are destroyed before the next seed lot is sown.

Preservation of wild *Arachis* species, in general, is difficult, particularly for accessions that produce a few seeds, and especially the section *Rhizomatosae* species, which are maintained as vegetative materials in greenhouse (Stalker and Simpson 1995). An international cooperative effort is underway to ensure that these vegetatively propagated species are maintained in multiple environments for conservation to minimize their loss (Singh and Simpson 1994). This effort involves the cooperation of USDA, North Carolina State University, Texas A&M University, ICRISAT, the Brazilian Corporation for Agricultural Research

Table 1.1 Major holdings of wild *Arachis* species accessions in genebank

Country	Institute	# Accessions
Argentina	Instituto Botánico del Nordeste, Universidad Nacional de Nordeste (IBONE)	109
Australia	Australian Tropical Crops and Forages Genetic Resources Centre	65
Brazil	Embrapa Recursos Geneticos e Biotecnologia (CENARGEN) Instituto AgronOmico de Campinas	450
Colombia	Centro Internacional de Agricultura Tropical (CIAT)	243
	Centro de Investigaciones de Nataima, Instituto Colombiano Agropecuario (ICA)	225
India	International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad	453
USA	USDA, Griffin, USA	498
	Texas A&M University, USA	798

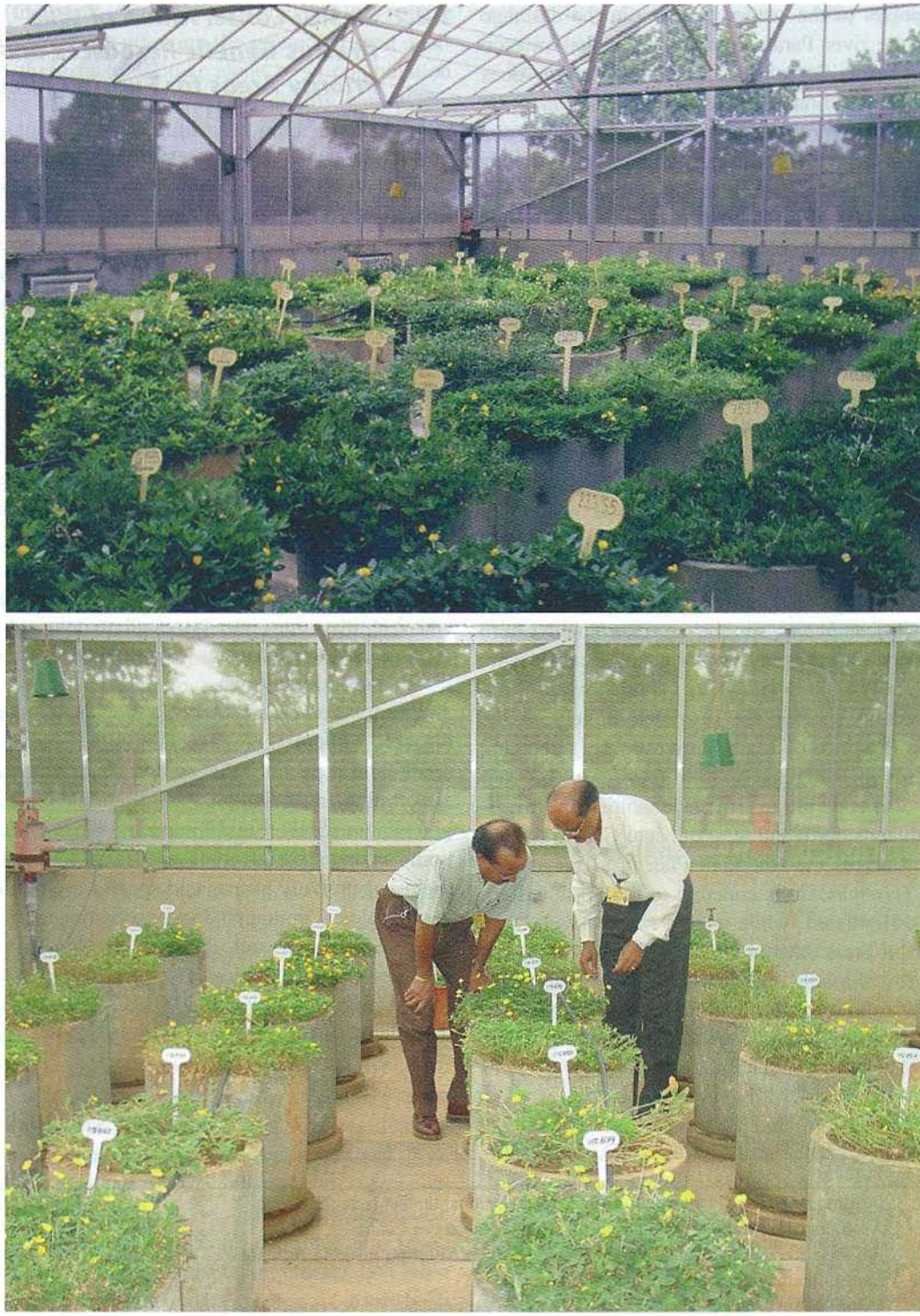

Fig. 1.1 (a) *Arachis* species grown in large cylindrical concrete structures in the *Arachis* House, ICRISAT, Patancheru, India. (b) Scientists examining wild *Arachis* species growing in these structures

(EMBRAPA), the Brazilian National Center for Genetic Resources and Biotechnology (CENARGEN), the Argentina National Institute of Agricultural Technology (INTA) and the Argentina Botanical Institute of the Northeast (IBONE).

Spatial analysis of in situ wild relatives distribution using species richness (areas potentially high in species richness), proximity to existing accessions (areas most distant from the existing collections, thus targeting geographical gaps in existing collections), proximity to protected areas (areas most distant from protected areas), and risk to genetic erosion (areas with the greatest risk of genetic erosion) revealed that hotspot regions for the wild *Arachis* species' richness in Brazil include Serra Geral de Goias, north-east of Brasilia, the region west of Campo Grande in Mato Grosso do Sul, and the region 170 km south of Cuiaba, in Mato Grosso. In addition, 300 km south-east of the city of Cuiabá, near Pedro Gomes, has also been identified as a species-rich area where species such as *A. cryptopotamica*, *A. diogoi*, *A. glabrata*, *A. helodes*, *A. hoehnei*, *A. kuhlmannii*, *A. lutescens*, *A. matiensis*, *A. stenosperma* and *A. subcoriacea* exist sympatrically (Jarvis et al. 2002, 2003). Another area is the municipality of Parauna in the state of Goias (Brazil), where only *A. prostata* and *A. glabrata* were collected in the past, as it is predicted that as many as six different species may be found in this region, although the land in this region is predominantly agricultural (Jarvis et al. 2003). Likewise, considering anthropogenic influences as a risk to genetic erosion, some areas in Bolivia, where about five species potentially lie sympatrically (Jarvis et al. 2002), have also been highlighted for future collections. Further, Jarvis et al. (2003) emphasized the need for more effort to collect and conserve species belonging to B-genome such as *A. williamsii*, *A. cruziana* and *A. ipaënsis* along with *A. martii*, *A. Pietrarellii*, *A. vallsii* and *A. monticola*, which are also under the risk of extinction.

1.2.3 Climate Change and Habitat Disturbances a Threat to Wild Arachis in South America

Climate change poses serious impacts on biodiversity and has a potential to wipe out biodiversity. Wild relatives of groundnut are at risk of extinction, threatening a valuable repository of genes needed for the improvement of the cultivated groundnut. In a recent study, it is predicted that in the next 50 years, as many as 61% of the 51 wild groundnut species studied would become extinct, as a result of climate change (Jarvis et al. 2008). The areas, where wild *Arachis* species are most at risk, include Santa Cruz to Cuiba and along the Andean fringe in the south of Santa Cruz (Bolivia), eastern Bolivia, Paraguay and south-western Brazil. In recent years, there have been intensive developmental activities in these regions, thus disturbing the remote and fragile environments (Jarvis et al. 2002). Most of the wild species generally occur in the region under intensive environmental disturbance, which has led to habitat destruction and genetic erosion. Some *Arachis* species are particularly threatened by habitat loss. The species, which are most restricted in distribution, include *A. archeri*, *A. setinervosa*, *A. marginata*, *A. hatschbachii*, *A. appressipila*, *A. villosa*, *A. cryptopotamica*, *A. helodes*, *A. magna* and *A. gracilis*. Their distribution is limited to less than 10,000 km^2 of climatically suitable wild habitat, while *A. burkatii*, *A. triseminata*, *A. tuberosa* and *A. Dardani* remain above 10,000 km^2, but their distribution has been reduced by more than 75% because of agricultural land use (Jarvis et al. 2003).

1.2.4 Ploidy Levels and Genome Variations Among Wild Arachis Species

The cultivated groundnut is a tetraploid with chromosome number, $2n = 40$, and genome size 2,813 Mbp. The first chromosome count reported for a wild species was $2n = 40$ for *A. glabrata* (Gregory 1946). Mendes (1947) published the chromosome count of $2n = 20$ for *A. oteroi*, *A. benthami*, *A. archeri*, *A. major* and *A. villosulicarpa*, which gave the first indication of the existence of $2n = 20$ and $2n = 40$ chromosomes in the genus *Arachis*. Later on, several studies confirmed the existence of $2n = 2x = 20$ and $2n = 4x = 40$, with basic chromosome number $n = 10$ (Krapovickas and Rigoni 1957; Krapovickas and Gregory 1960; Conagin 1964; Smartt 1965; also see Table 1.2). Polyploidy has apparently arisen independently at least twice in the genus, in the sections *Arachis* and *Rhizomatosae*. In the section *Arachis*,

Table 1.2 Chromosome counts in species belonging to the genus *Arachis* (Krapovickas and Gregory 1994; Valls and Simpson 2005)

Species	Chromosome number (2*n*)	Species	Chromosome number (2*n*)
Section *Trierectoides*			
A. guaranitica	20	*A. tuberosa*	20
Section *Erectoides*			
A. martii	20	*A. gracilis*	20
A. brevipetiolata	20	*A. hermannii*	20
A. oteroi	20	*A. archeri*	20
A. hatschbachii	20	*A. stenophylla*	20
A. cryptopotamica	20	*A. paraguariensis* spp. *capibarensis*	20
A. major	20	*A. paraguariensis* spp. *paraguariensis*	20
A. benthamii	20	*A. porphyrocalyx*	20
A. douradiana	20		
Section *Extranervosae*			
A. setinervosa	20	*A. retusa*	20
A. macedoi	20	*A. burchellii*	20
A. marginata	20	*A. pietrarellii*	20
A. prostrata	20	*A.villosulicarpa*	20
A. lutescens	20	*A. submarginata*	20
Section *Triseminatae*			
A. triseminata	20		
Section *Heteranthae*			
A. giacomettii	20	*A. dardani*	20
A. sylvestris	20	*A. interrupta*	20
A. pusilla	20	*A. seridoënsis*	20
Section *Caulorrhizae*			
A. repens	20	*A. pintoi*	20
Section *Procumbentes*			
A. lignosa	20	*A. appressipila*	20
A. kretschmeri	20	*A. vallsii*	20
A. rigonii	20	*A. subcoriacea*	20
A. chiquitana	20	*A. hassleri*	20
A. matiensis	20	*A. pflugeae*	20
Section *Rhizomatosae*			
A. burkartii	20	*A. glabrata* var. *glabrata*	40
A. pseudovillosa	40	*A. glabrata* var. *hagenbeckii*	40
A. nitida	40		
Section *Arachis*			
A. glandulifera	20	*A. decora*	18
A. cruziana	20	*A. herzogii*	20
A. monticola	40	*A. microsperma*	20
A. magna	20	*A. villosa*	20
A. ipaënsis	20	*A. helodes*	20
A. valida	20	*A. correntina*	20
A. williamsii	20	*A. simpsonii*	20
A. batizocoi	20	*A. cardenasii*	20
A. duranensis	20	*A. kempff-mercadoi*	20
A. hoehnei	20	*A. diogoi*	20
A. stenosperma	20	*A. kuhlmannii*	20
A. praecox	18	*A. gregoryi*	20
A. palustris	18	*A. krapovickasii*	20
A. benensis	20	*A. linearifolia*	20
A. trinitensis	20	*A. schininii*	20
		A. hypogaea	40

A. monticola and *A. hypogaea* are, therefore, tetraploids, which have attained diplontic behavior, though they sometimes show secondary associations in the form of quadrivalents and trivalents (Singh and Moss 1982). In addition to the basic chromosome number $n = 10$ found in most of the diploid and tetraploid species in all the nine sections of the genus *Arachis*, the basic chromosome number $n = 9$ has also been found in four diploid species, *A. palustris* (Lavia 1996), *A. praecox* (Lavia 1998), and *A. decora* (Penaloza et al. 1996) of section *Arachis* and *A. porphyrocalyx* of section *Erectoides* (Penaloza and Valls 2005). It has been suggested that the $n = 9$ constitutes a derived number from $n = 10$ (Lavia 1998); however, the cytogenetic mechanism involved in its origin is not yet known with certainty.

Smartt et al. (1978a) identified two genomes (A and B) in section *Arachis*, both of which occur in cultivated groundnut (*A. hypogaea*) and the tetraploid wild species *A. monticola*, which was further supported by later studies in the two-genome theory in cultivated groundnut, following the chromosome analysis (Stalker and Dalmacio 1981; Singh and Moss 1982). Subsequently, using chromosome morphology and crossing relationships, three genomes (A, B and D) were proposed in section *Arachis* diploid species (Smartt 1965; Smartt et al. 1978a; Singh and Moss 1982, 1984a, b; Stalker 1991). The A-genome is characterised by a pair of chromosomes smaller than the other chromosomes, while the B-genome lacks this smaller chromosome pair. Most diploid wild species contain the A-genome. Only a single B-genome species *A. batizocoi* was initially recognized, but now several others have been identified (Fernandez and Krapovickas 1994). The only D-genome diploid species is *A. glandulifera*, native to eastern Bolivia (Stalker 1991). "A"-genome species show considerable variation in fertility levels among the progenies from the crosses within A-genome species (Smartt 1965; Gregory and Gregory 1979; Spielman et al. 1979; Stalker and Wynne 1979; Singh and Moss 1982, 1984a). Subsequently, using the crossing relationships that Gregory and Gregory (1979) initiated, Smartt and Stalker (1982) proposed a series of genomes for diploid species in the genus *Arachis*, which include the following:

A = section *Arachis*, perennials and most annuals
B = section *Arachis* (*A. batizocoi*)
D = section *Arachis* (*A. glandulifera*)

Am = section *Ambinervosae*
C = section *Caulorrhizae*
E = section *Erectoides* (subgenomes E_1, E_2, E_3, corresponding to series)
Ex = section *Extranervosae*
T = section *Triseminatae*
R_1 = section *Rhizomatosae*, series Prorhizomatosae

The nuclear DNA content has an important function in the evolution and adaptation of the plants (Price 1976; Bennett 1982). Lavia and Fernández (2008) studied the genome size of 16 species of *Arachis* with $n = 10$ and three with $n = 9$, involving both diploid and tetraploid species. DNA content (2C) between all diploid species of *Arachis* with $2n = 20$ varied from 2.87 pg in *A. retusa* to 6.59 pg in *A. douradiana*. Likewise, the DNA content in species with $2n = 18$ varied from 3.26 pg in *A. palustris* to 4.16 pg in *A. decora*. The species with greater DNA contents have the longest chromosomes, while those with lower DNA contents have smaller chromosomes. In contrast, DNA content in *A. hypogaea* ($2n = 40$) ranged between 10.87 and 11.92 pg. These results suggest that in the evolution of *Arachis* genome, both increases and diminution of DNA content would have occurred. Species with greater DNA content are included in sections believed to have a more recent origin, whereas those that contain lower DNA content belong to the oldest section, suggesting genome evolution of *Arachis* towards higher DNA content. Reduction of the DNA content after polyploidization would have happened in *A. hypogaea* (Lavia and Fernández 2008).

1.2.5 Crossing Relationships Among Wild Arachis Species

Gregory and Gregory (1979) reported successful intrasectional hybrids in sections *Arachis*, *Erectoides*, *Rhizomatosae*, *Caulorrhizae*, *Extranervosae*, *Triseminatae* and *Ambinervosae* and the intersectional hybrids involving *Arachis* with *Erectoides* and *Rhizomatosae*; *Erectoides* with *Rhizomatosae*, *Caulorrhizae* and *Ambinervosae* and *Ambinervosae* with *Extranervosae*, which led to the establishment of intra- and intersectional crossing relationships between the nine sections of the genus *Arachis* (Krapovickas and Gregory 1994). No successful intersectional cross of the diploid annual wild *Arachis* species belonging to section *Arachis* were obtained with those in section *Triseminatae*, *Rhizomatosae* (*A. burkartii*), and with perennials and tetraploid species of the section *Arachis* (Krapovickas and Gregory 1994). A very high level of genetic isolation was found among the sections *Erectoides*, *Trierectoides*, *Extranervosae*, *Triseminatae* and *Heteranthae*, confirming their primitiveness in the genus *Arachis*, which has been further supported by the comparative morphology of the "B" (SAT) chromosome and the absence of the "A" pair (Fernandez and Krapovickas 1994). On the basis of taxonomic and cross-compatibility studies, Krapovickas and Gregory (1994) suggested that *Trierectoides*, *Erectoides*, *Extranervosae*, *Triseminatae* and *Heteranthae* are the oldest sections while *Procumbentes*, *Caulorrhizae*, *Rhizomatosae* and *Arachis* are of more recent origin. Intersectional hybrids involving section *Arachis* with *Rhizomatosae*, *Extranervosae*, *Procumbentes* and *Erectoides* have also been successful at ICRISAT (Mallikarjuna and Bramel 2001; Mallikarjuna 2002, 2005).

1.3 Taxonomy and Species Diversity of Wild *Arachis* Species

Krapovickas and Gregory (1994) used 32 descriptors, mostly morphological traits, to study taxonomy of 69 *Arachis* species. Using taxonomy and crossing incompatibility studies, they classified 69 species to nine sections and suggested that *Trierectoides*, *Erectoides*, *Extranervosae*, *Triseminatae* and *Heteranthae* are the oldest sections while *Procumbentes*, *Caulorrhizae*, *Rhizomatosae* and *Arachis* are of more recent origin. Valls and Simpson (2005) described 11 new species (*A. porphyrocalyx*, *A. submarginata*, *A. pflugeae*, *A. hassleri*, *A. interrupta*, *A. seridoënsis*, *A. nitida*, *A. linearifolia*, *A. shcininii*, *A. gregoryi* and *A. krapovickasii*) of *Arachis*, representing seven of the nine taxonomic sections of the genus. Of these, eight were earlier classified in Krapovickas and Gregory (1994) monograph, but are now treated with their own specific epithet. Thus, the description of these 11 species will help clarify the systematics of the genus *Arachis*, as well as aid in understanding the evolutionary pathway of certain important materials. Some of these may have played a role in developments that led to the origin of cultivated groundnut. The key morphological

features that distinguish these species include growth habit (procumbent, erect, prostrate and decumbent), types of leaves (trifoliate and tetrafoliate), plant type (rhizomatous and nonrhizomatous), leaflet shape, leaflet surface, leaflet length and width, petiole length, leaflet margins, presence or absence of bristles on stipules, petiole and leaflet surface, standard petal and wing color and stem and peg characteristics (Krapovickas and Gregory 1994, 2007; Valls and Simpson 2005). Further, the species in sections *Arachis* and *Rhizomatosae* are characterized by short pegs that grow vertically in comparison to the species in the other seven sections in which the pegs are very long and superficial.

A. Pintoi is a herbaceous perennial species grown for multipurpose use, ranging from use as forage, ground cover in fruit orchards, forest and low tillage system, erosion control, and ornamental purposes. Carvalho and Quesenberry (2009) characterized *A. Pintoi* accessions for phenotypic diversity, which represented great morphological variability. Of the 595 correlations computed, 96 were statistically significant. They detected biologically meaningful correlations ($r^2 = 0.50$) for leaf length and pod weight, leaf length and pod width, leaf length and seed weight and leaf length and seed width. Total genetic diversity in this study was 0.71, with both principal component and cluster analysis differentiating the accessions into four distinct groups. Researchers at ICRISAT have characterized 267 wild *Arachis* accessions of 37 species for 33 qualitative and 15 quantitative traits (Table 1.3) at *Arachis* house, wherein six plants of each of the 267 accessions were grown under large-size cylindrical concrete structures. Preliminary results revealed that species exhibited 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, with Shannon-Weaver diversity index ranging 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 (Upadhyaya unpublished data).

Unlike cultivated groundnut germplasm, the evaluation of wild relatives in the field is not feasible because of their long generation time (from annual to perennial life cycle), extensive ground coverage, and thus the chance of mixing with other accessions, and risk of leftover pods/seeds remaining deep in the soil after harvest, thus becoming a source of contamination for the next crop. Researchers have, therefore, used isozyme and hybridization- and PCR-based markers to assess the intra- and interspecific variation, which have revealed high variability among wild *Arachis* species. The variability and relationship among 15 accessions of *A. glabrata* were studied by using isozymes (Maass and Ocampo 1995). In this study, polyacrylamide gel electrophoresis (PAGE) was applied to rhizome-tip tissue, which showed a high degree of intraspecific polymorphism for the isozymes α-EST, ACP, GOT and DIA. The four isozyme systems differentiated all the 15 accessions of *A. glabrata*. Using restriction fragment length polymorphism (RFLP) markers, Gimenes et al. (2002) analyzed four A-genome species (*A. cardenasii*, *A. correntina*, *A. duranensis*, *A. kempff-mercadoi*), three B-genome species (*A. batizocoi*, *A. ipaënsis*, *A. magna*), the AABB allotetraploid *A. hypogaea* and introgression lines resulting from a cross between *A. hypogaea* and *A. cardenasii*. All the *A. batizocoi* accessions were clustered in a separate group, suggesting that this species is not closely related to *A. hypogaea*, *A. ipaënsis* or the A-genome species analyzed. The highest level of genetic variation was found in *A. cardenasii* indicating that all accessions of wild species of *Arachis* might not be autogamous, as reported for *A. hypogaea* (Gimenes et al. 2002). Nóbile et al. (2004) evaluated genetic variability within and among accessions of wild *Arachis* species, *A. glabrata*, *A. burkartii*, *A. pseudovillosa* and *A. nitida* belonging to the section *Rhizomatosae* using *random amplified polymorphic DNA* (*RAPD*) markers that detected the highest genetic variation in diploid species *A. burkartii*. The diploid species *A. burkartii* and the tetraploid species *A. glabrata*, *A. pseudovillosa* and *A. nitida* were grouped separately, suggesting that none of these tetraploid species originated from *A. burkartii*. Hoshino et al. (2006) used heterologous simple sequence repeat (SSR) markers to characterize genetic diversity among 76 accessions of 34 species from nine sections of the genus *Arachis*. The total number of alleles ranged from 28 in *A. tuberosa* (section *Trierectoides*) to 81 in *A. paraguariensis* (section *Erectoides*). All the species investigated showed high polymorphism among their accessions; however, accessions were not grouped exactly according to the species and sections to which they belonged. This difference may be attributed to the high polymorphism

Table 1.3 List of descriptors used for characterizing wild *Arachis* species accessions at ICRISAT, Patancheru, India

Qualitative descriptor

Growth habit (GH); root growth (RG); stem modification (SM: absent, rhizome and stolon); branching pattern (BP: alternate 2:2:2, alternate 2:1:2 or 1:2:1, sequential, irregular); pigmentation on main stem (PMS: absent, present), main stem hairiness [MSH: Glabrous, Subglabrous (hairs in one or two rows), Moderately hairy (hairs in 3–4 rows), Very hairy (stem surface mostly covered with hairs), wooly (villous hairs >2 mm)]; main stem hair type [MSHT: Glandular (bristles), Non-glandular, or both types]; flowers on main stem (FLM); hypanthium hairiness [HYH: Glabrous, Hairy, wooly (villous hairs >2 mm)]; standard petal color [SPC: White (155 A–D), Lemon yellow (6 A–B), Yellow (14 A–B), Orange-yellow/yellow-orange (24 B), Orange (24 A or 25 A), Dark orange (28 A), Garnet/brick red/reddish orange (35 A)]; standard petal markings on front face [SPMFF: Absent, Lemon yellow (6 A–B), Yellow (14 A–B), Orange-yellow/yellow-orange (24 B), Orange (24 A or 25 A), Dark orange (28 A), Garnet/brick red/reddish orange (35 A), Purple]; standard petal markings on back face (SPMBF: Absent, Red or purple blush, Red or purple streaks, Grayed orange streaks, and Greenish purple streaks); wing petal color (WPC: Yellow, lemon yellow, orange, yellow and white); leaflet surface on main stem (LSMS: Non-shiny, shiny); leaflet surface on primary lateral (LSPL: Non-shiny, shiny); leaflet color (LC) on main stem and on primary laterals [Yellow/yellow-green (146 A–D), light green (137 A–D), green (139 A–B), dark green (131 A), bluish green and purplish green]; leaflet shape on apical main stem (LSAMS: Cuneate, obcuneate, wide-elliptic, narrow-elliptic, elliptic, suborbicular, orbicular, ovate, obovate, oblong, oblong-lanceolate, lanceolate, ob-lanceolate, linear-lanceolate, others); leaflet shape on apical primary lateral (LSAPL: same as described for LSAMS); leaflet shape on basal main stem (LSBMS: same as described for LSAMS); leaflet shape on basal primary lateral (LSBPL: same as described for LSAMS); leaflet hairiness (LH) on main stem [Glabrous; almost glabrous on both surfaces; almost glabrous above, hairy below; almost glabrous below, hairy above; hairy on both surfaces; wooly(villous hairs >2 mm)]; leaflet hairiness on primary lateral (LHPL: same as described for LH); leaflet bristle (LB) on main stem (Absent, bristles on upper surface, bristles on lower surface, bristles on both surface); leaflet bristle on primary lateral (LBPL: Absent, bristles on upper surface, bristles on lower surface, bristles on both surface); leaflet hairiness on margin of main stem and primary laterals [LHMSPL: Absent, ciliate, wooly (villous hairs >2 mm)]; leaflet bristle on margin of main stem and primary laterals (LBMSPL: Absent, few, setose); leaflet midrib hairiness on upper main stem (LMHUMS: Glabrous, subglabrous, hairy); leaflet midrib hairiness on upper primary lateral (LMHUPL: Glabrous, subglabrous, hairy); leaflet midrib hairiness on lower main stem (LMHLMS: Glabrous, subglabrous, hairy); leaflet midrib hairiness on lower primary lateral (LMHLPL: Glabrous, subglabrous, hairy); leaflet tip shape of the main stem (LTSMS: Acuminate, acute, indented, mucronate, obtuse); leaflet tip shape of primary lateral (LTSPL: Glabrous, subglabrous, hairy); nature of stipule on primary lateral (NSTPL: Open, partially open, tubular); stipule hairiness on outside of the main stem [SHOMS: Glabrous, Subglabrous, Hairy, Very hairy, wooly (villous hairs >2 mm)]; stipule hairiness on outside primary lateral [SHOPL: Glabrous, Subglabrous, Hairy, Very hairy, wooly (villous hairs > 2mm)]; stipule hairiness on margin of the main stem [SHMMS: Glabrous, Subglabrous, Hairy, Very hairy, wooly (villous hairs >2 mm)]; stipule hairiness on margin of primary lateral branch [SHMPLB: Glabrous, Subglabrous, Hairy, Very hairy, wooly (villous hairs >2 mm)]; stipule bristles outside of the main stem (SBOMS: absent, a few, many); stipule bristle outside primary lateral branch (SBOPLB: absent, a few, many); stipule bristles on margin of the main Stem (SBMMS: absent, a few, many); stipule bristle on the margin of primary lateral (SBMPL: absent, a few, many); nature of petiole on main stem and on primary laterals (NPMSPL: Straight, slightly reflexed, reflexed); petiole hairiness on main stem [PHMS: Glabrous, subglabrous, hairy, very hairy, wooly (villous hairs >2 mm)]; petiole hairiness on primary lateral [PHPL: Glabrous, subglabrous, hairy, very hairy, wooly (villous hairs >2 mm)]; petiole bristle on main stem (PBMS: Absent, few, many); petiole bristle on primary lateral (PBPL: Absent, few, many); petiole groove on main Stem [PGMS: Absent (0%), shallow (<15%), deep (16–30%), very deep (>30%)]; petiole groove on primary lateral [PGPL: Absent (0%), shallow (<15%), deep (16–30%), very deep (>30%)]; rachis groove on main stem [RGM: Absent (0%), shallow (<15%), deep (16–30%), very deep (>30%)]; rachis groove on primary lateral [RGPL: Absent (0%), shallow (<15%), deep (16–30%), very deep (>30%)]; peg growth (PG: Almost horizontal, almost vertical, twisted); peg pigmentation (PGP: absent, present); pod beak (PB: Absent, slight, moderate, prominent, very prominent); pod reticulation (PR: Smooth, slight, moderate, prominent, very prominent); seed color [SC: Off-white (158 A–D and 159 C–D), tan (173 C–D and 174 C–D)]; number of segments between pods (NSBP: 1 segment, 2 segments, 1–2/2–1 segments, 1–2–3 segments, 2–3 segments, 3 segments)

Quantitative descriptor

Days to emergence (DE); days to 50% flowering (DF); upper lip calyx lobation [ULCL: indentation and number of lobes of the upper lip of calyx of flowers recorded in 4 classes (entire, 2 lobes, 3 lobes, 4 lobes) 4–6 months after emergence], number of lateral branches (NLB); standard petal length (SPL); standard petal width (SPW); leaflet length of the apical primary lateral (LLAPL); leaflet width of apical main stem (LWAMS); leaflet width of apical primary lateral (LWAPL); leaflet length on basal main stem (LLBMS); leaflet length on basal primary lateral (LLBPL); leaflet width on basal main stem (LWBMS); leaflet width on basal primary lateral (LWBPL); stipule length on main stem (SLMS); stipule length on primary lateral branch (SLPLB); stipule adnation length on main stem (SALMS: measured as length of adnate part of stipule of fourth leaf); stipule adnation length on primary lateral branch (SALPLB); stipule adnation width on main stem (SAWMS); stipule adnation width on primary lateral branch (SAWPLB); petiole length on main stem (PLMS); petiole length of primary lateral (PLPL); rachis length of the main stem (RLMS); rachis length of primary lateral (RLPL); main stem height (MSH); main stem thickness (MST); days to maturity (DM), peg length (PL); basal segment length (BSL); apical segment length (ASL); pod length (PDL); pod width (PDW), length of first isthmus (LFI); seed length (SDL); seed width (SDW), 100 seed weight (HSW)

found in some of the loci and sharing of alleles among species from different sections. Gimenes et al. (2007) reported high transferability of microsatellite markers of *A. hypogaea* to other species of the genus, and identified two groups – the first consisting of *A. hypogaea*, *A. monticola* and all the analyzed A-genome species while the second contained B- and D-genome species. Mallikarjuna et al. (2007) studied the genetic relationship among two *A. diogoi* accessions and three *A. chiquitana* accessions, using SSRs and high-throughput assay. Two *A. diogoi* accessions, ICG 4983 and ICG 8962, and the two *A. chiquitana* accessions, ICG 13181 and ICG 13241, formed two distinct groups. The third *A. chiquitana* accession, ICG 11560, did not group closely with the other *A. chiquitana* accessions, but showed a closer relationship with them than with the *A. diogoi* accessions. These results showed that *A. chiquitana* accessions, particularly ICG 11560, are not related to the accessions of *A. diogoi* and that the accessions belonging to these two species are different.

Angelici et al. (2008) used SSRs to study the genetic diversity among 77 accessions of the four species from section Rhizomatosae, the diploid *A. burkartii* and the tetraploid *A. glabrata*, *A. pseudovillosa* and *A. nitida*. The 15 SSR loci detected 249 alleles and high degrees of intra- and interspecific polymorphism. The diploid accessions grouped in one cluster and the tetraploid accessions in another cluster. The markers differentiated all the 77 accessions but the genetic distance could not be correlated with geographic origin. Furthermore, Robledo and Seijo (2008) studied the genomic affinities of *A. glandulifera* with A- and B-genome by comparing several chromosome landmarks and by total genome hybridization, using fluorescence in situ hybridization (FISH) of the 5S and 45S rRNA genes and heterochromatic 4′-6′-diamidino-2-phenylindole (DAPI) positive bands. Their results revealed very poor homologies with all the A- and B-genome taxa, supporting the special genome constitution (D-genome) of *A. glandulifera*. In a study involving 14 wild *Arachis* species from different sections and 24 allotetraploid groundnut cultivars from several countries and belonging to different botanical types, Tang et al. (2008) revealed that groundnut cultivars were closely related to each other, and shared a large number of alleles. In contrast, the species in genus *Arachis* shared few alleles. Further, the cultivars in this study could be partitioned into two main groups and four subgroups at the molecular level, and that *A. duranensis* is one of the wild ancestors of *A. hypogaea*. The lowest genetic variation was detected between *A. cardenasii* and *A. batizocoi*, and the highest between *A. pintoi* and the species in the section *Arachis*. This study also revealed that accessions in section *Heteranthae* were closest to the tested accessions in section *Arachis*, followed by the tested accessions in the sections *Procumbentes*, *Rhizomatosae* and *Caulorrhizae*, respectively, thus providing breeders insights into the use of wild species out of section *Arachis*, for the improvement of cultivated groundnut. At ICRISAT, 47 accessions of 14 wild *Arachis* species along with 805 accessions of cultivated *A. hypogaea* (322 accessions of *hypogaea* type and 483 of *fastigiata* type) were genotyped, using 21 SSR markers. The common alleles were higher in the wild *Arachis* species (359) than in the cultivated *fastigiata* (230) and *hypogaea* (190) types. Wild species also possessed the highest number of unique alleles (101), and the gene diversity was 0.870, ranging from 0.434 to 0.947. The wild *Arachis* accessions shared only 15 alleles with the subspecies *hypogaea* and 32 alleles with the subspecies *fastigiata*.

1.4 Wild *Arachis* as Source of Variation for Agronomic Traits

Wild *Arachis* species harbor very high levels of resistance to many biotic and abiotic stresses when compared with cultivated groundnut (Dwivedi et al. 2003, 2008; Table 1.4). Examples include resistance to rust, early leaf spot, late leaf spot, nematode, peanut mottle virus, peanut stripe virus, peanut bud necrosis virus, tomato-spotted wilt virus, groundnut rosette disease, aflatoxin, corn ear worm, southern corn root worm, thrips, leaf hoppers and *Spodoptera*. Further, the mechanism and genetic control of resistance in wild relatives appear to be different than that in cultivated types. For example, resistance to rust in crosses involving wild relatives is partially dominant (Singh et al. 1984). Sharma et al. (2003) found several morphological traits associated with tolerance to insect pests. For example, main stem thickness and hairiness, hypanthium length, leaflet shape and length, leaf hairiness, standard petal length and petal markings, basal leaflet width, stipule adnation length and width, and peg length showed significant correlation with damage by

Table 1.4 Wild *Arachis* species resistant to pests and diseases

Species	Trait	References
A. hagenbeckii, *A. glabrata* and *A. repens*	Early leaf spot (ELS)	Gibbons and Bailey (1967)
A. diogoi and *A. cardenasii*	ELS and late leaf spot (LLS)	Abdou et al. (1974)
A. glabrata	Peanut mottle virus (PMV)	Demski and Sowell (1981)
A. chacoense, *A. cardenasii*, *A. stenosperma*, *A. repens*, *A. appressipila*, *A. paraguariensis*, *A. villosulicarpa*, *A. hagenbeckii*, *A. glabrata*, *A. batizocoi*, *A. duranensis*, *A. correntina*, *A. villosa* and *A. pusilla*	LLS, rust	Subrahmanyam et al. (1983, 1985a)
A. pusilla, *A. cardenasii*, *A. diogoi* and *A. correntina*	PMV, tomato-spotted wilt virus (TSWV)	Subrahmanyam et al. (1985b)
A. monticola	ELS	Subrahmanyam et al. (1985c)
A. batizocoi and *A. cardenasii*	Nematode	Nelson et al. (1989) and Holbrook and Noe (1990)
A. cardenasii, *A. chacoense* and *A. stenosperma*	ELS, LLS, rust	Nigam et al. (1991)
A. cardenasii and *A. duranensis*	Seed colonization and aflatoxin production	Nigam et al. (1991)
A. chacoense and *A. pusilla*	PMV	Nigam et al. (1991)
A. cardenasii	Peanut stripe virus (PStV)	Nigam et al. (1991)
A. correntina, *A. chacoense*, *A. stenosperma* and *A. villosulicarpa*	Insect-pests	Nigam et al. (1991)
A. chacoense	ELS, TSWV, rust, nematode, thrips, corn earworm (CEW), leaf hoppers	Stalker (1992)
A. cardenasii, *A. stenosperma* and *A. batizocoi*	LLS, TSWV, ELS, rust, nematode, CEW, leaf hoppers	Stalker (1992)
A. helodes, *A. sylvestris*, *A. kretschmeri*, *A. kuhlmannii* and *A. stenosperma*	Nematode	Sharma et al. (1999)
A. benensis, *A. cardenasii*, *A. villosa*, *A. appressipila* and *A. triseminata*	Peanut bud necrosis virus	Reddy et al. (2000)
A. appressipila, *A. triseminata*, *A. magna*, *A. sylvestris*, *A. pusilla*, *A. valida* and *A. dardani*	ELS	ICRISAT (2000)
A. hoehnei, *A. duranensis* and *A. kuhlmannii*	LLS, rust	Pande and Rao (2001)
A. diogoi, *A. hoehnei*, *A. kretschmeri*, *A. appressipila*, *A. cardenasii*, *A. villosa*, *A. stenosperma*, *A. pintoi*, *A. kuhlmannii*, *A. triseminata* and *A. decora*	Groundnut rosette disease	Subrahmanyam et al. (2001)
A. cardenasii	Rust, ELS, nematode, southern corn rootworm, leaf hopper	Stalker et al. (2002a, b) and Stalker and Lynch (2002)
A. cardenasii, *A. duranensis*, *A. kempff-mercadoi*, *A. monticola*, *A. stenosperma*, *A. paraguariensis*, *A. pusilla* and *A. triseminata*	Leaf miner, *Helicoverpa*, leaf hopper, rust, LLS	Sharma et al. (2003)
A. kempff-mercadoi	ELS, LLS, *Spodoptera*	Mallikarjuna et al. (2004)

Helicoverpa armigera, *Spodoptera litura* and leafhoppers. Wild relatives are also reported to possess high oil and protein content (Dwivedi et al. 2003). Several *Arachis* species are extremely drought tolerant (Stalker and Moss 1987). At ICRISAT, 282 wild *Arachis* accessions belonging to 38 species were evaluated for soil plant analysis development (SPAD), chlorophyll meter readings (SCMR) and specific leaf area (SLA) traits related to drought tolerance at two stages, viz., 60 days after sowing (DAS) and 80 DAS. Enormous variability was observed among the accessions for these two traits, which ranged from 26.41 to 62.38 for SCMR at 60 DAS and 29.01 to 60.28 at 80 DAS, 39.23 to 357.70 for SLA at 60 DAS and 91.53 to 209.39 at 80 DAS (Upadhyaya unpublished data). More recently, Nautiyal et al. (2008) reported wide genetic variability in leaf characteristics such as color, shape, hairiness, specific leaf area (SLA, length, width and thickness) among wild *Arachis* species, that were associated with cold and heat tolerance as measured by relative leaf

injury (RI). The SLA in 36 wild *Arachis* accessions ranged from 66 to 161 $cm^2\ g^{-1}$. Using RI as the measure of tolerance, *A. glabrata* 11,824 and *A. paraguariensis* 12,042 were identified as heat and cold tolerant, respectively, while *A. appresipila* 11,786 was found to be susceptible to both heat and cold stress. Further, when detecting the concentration of various leaf constituents, the total protein, phenols, sugars, reducing sugar, amino acids, proline, epicuticular wax load, and chlorophyll were found to vary significantly among heat- and cold-tolerant accessions. For example, the epicuticular wax load ranged between 1.1 and 2.5 mg dm^2 among 13 *A. glabrata* accessions. The high-wax accessions showed a higher diffusion resistance (dr) as compared to low-wax type; though the transpiration rate (tr) in high-wax type was moderate (between 9.5 and 11.6 $\mu g\ cm^{-2}\ s^{-1}$). These accessions also showed large genetic variation in canopy temperature as well. For example, the fully turgid leaves with relative water content $\geq 91\%$ showed leaf water potential (ψ_{leaf}) between -0.7 and -1.2 MPa. These results revealed that plants with thicker leaves are better protected from heat injuries while epicuticular wax load helps in maintaining stomotal regulation and leaf water relations, thus affording adaptation to wild *Arachis* species to thrive under water-limited environments. The genetic upgradation of the cultivated groundnut necessitates the use of wild *Arachis* gene pools to expand its genetic variability.

1.5 Barriers to Interspecific Hybridization

Many of the wild *Arachis* species are not cross-compatible with cultivated groundnut. The major barrier for gene introgression is postzygotic failure of embryo development. Researchers have used a number of techniques to either circumvent or overcome barriers to hybridization, which include the use of hormonal treatment to overcome pre- and postfertilization barriers or embryo rescue, if postfertilization barriers exist.

The species in the secondary gene pool, which is represented by the diploid species of the section Arachis, have greater potential as they possess very high levels of resistance to many pests and diseases. However, utilization of the secondary gene pool for the introgression of useful genes into *A. hypogaea* shows sterility barriers due to different ploidy levels, genomic incompatibilities and cryptic genetic differences, which could be restored by manipulating ploidy levels, as discussed in Sect. 1.10.

Direct intersectional hybridization with *A. hypogaea* has been difficult, necessitating the use of hormonal treatment to overcome pre- and postfertilization barriers or embryo rescue, if postfertilization barriers exist, which may, to a large extent, be overcome using in vitro techniques such as cell and protoplast culture, ovule and embryo culture or both. For example, in vitro culture of ovules or embryos has been successfully used to produce intersectional hybrids in many genera (Narayanaswami and Norstog 1964; Collins et al. 1984). However, several factors including genotypic specificity, media composition, concentrations of growth hormones and environmental conditions alone or in combinations influence the successful use of ovule and embryo culture techniques in interspecific crosses particularly with species from more distant gene pool (Sastri and Moss 1982; Mallikarjuna and Sastri 1985a, b). The intersectional hybrid between *A. hypogaea* and *A. glabrata* has been developed following embryo rescue technique and the resultant hybrid inherited the resistance to rust, late leaf spot, peanut bud necerosis and peanut stripe diseases from the pollen parent *A. glabrata* (Mallikarjuna 2002; Mallikarjuna and Sastri 2002). Likewise, using hormonal application to the pollinated pistil followed by embryo rescue technique, Mallikarjuna (2005) produced the first fertile intersectional hybrid between *A. hypogaea* and *A. chiquitana* of section *Procumbentes*. *A. chiquitana* is reported resistant to seed colonisation by *Aspergillus flavus*. Clearly, these studies demonstrate that it is possible to access the desirable traits across the sections for broadening the genetic base of cultivated groundnut by following various approaches.

1.6 Genomic Resources to Monitor Introgression in Interspecific Crosses Involving Wild *Arachis* Species

The genetic linkage maps based on interspecific crosses will be useful in locating specific genes of interest in the interspecific progenies that provide

a way to accomplish interspecific gene transfer with minimum linkage drag, thus improving the prospects for successful introgression of desirable genes from wild relatives (Tanksley et al. 1989; Tanksley and McCouch 1997). Halward et al. (1993) were the first to report RFLP-based genetic linkage map, involving 87 F_2 population of the cross involving diploid *Arachis* species *A. stenosperma* and *A. cardenasii*, with a total map distance of 1,063 cm, which contained 117 RFLP loci on 11 linkage groups. Burow et al. (2001) used BC_1F_1 population (78) derived from synthetic amphiploid TxAG-6 (Simpson et al. 1993) and Florunner to develop the first RFLP-based tetraploid genetic map, which mapped 370 RFLP loci on 23 linkage groups (LGs) with a total map distance of 2,210 cm. Subsequently, Moretzsohn et al. (2005) constructed the first SSR-based genetic map of *Arachis* by using F_2 population, involving AA-genome diploid species *A. duranensis* and *A. stenosperma*, which mapped 170 SSR loci on 11 LGs covering 1,231 cm and average marker density of 7.24 cm. Gobbi et al. (2006) mapped 130 SSR loci on 10 LGs involving diploid B-genome donor species, *A. ipaënsis* and *A. magna*. It is expected that with the availability of AA- and BB-genome-based genetic maps for *Arachis*, it would be possible to use these segregating loci to tag gene of interest in interspecific crosses. The group in Brazil now uses synthetic amphidiploids to construct a reference map, which they are using to access the near-complete genome sequences of model legumes (*Medicago truncatula* and *Lotus japonicus*), in a way that would enhance understanding of the *Arachis* genome. To do that, they placed more than 80 legume anchor markers (Fredslund et al. 2006) on the AA-genome map and analyzed the synteny between *Arachis* and the model legumes, identifying affinities in nine of the ten *Arachis* linkage groups and model legume chromosomes, some showing substantial regions of marker colinearity (Moretzsohn et al. 2007). The combination of SSR-based genetic maps of diploid species and synthetic amphiploids incorporating various exotic genomes would unlock the hidden treasure in wild *Arachis* species and would facilitate the marker-assisted introgression of important traits into the cultivated groundnut.

Guimarães et al. (2008) constructed and characterized two large-insert bacterial artificial chromosome (BAC) libraries, one for each of the diploid ancestral species. The libraries (AA and BB) are ca. 7.4 and ca. 5.3 genome equivalents, respectively, with low organelle contamination and average insert sizes of 110 and 100 kb. These diploid BAC libraries are important tools for the isolation of wild alleles conferring resistances to biotic stresses, comparisons of orthologous regions of the AA and BB-genomes with each other and with other legume species and will facilitate the construction of a physical map.

Garcia et al. (1995) showed introgression of genes from *A. cardenasii* into *A. hypogaea* in 10 of 11 LGs, which they used to enhance the selection efficiency for developing nematode-resistant germplasm (Garcia et al. 1996). Burow et al. (1996) identified two random amplified polymorphic DNA (RAPD) markers linked with nematode resistance in BC_4F_2 population of the cross Florunner × TxAg-6 that were closely linked to each other. One marker RKN229 was 9 cm away from resistance locus (Burow et al. 1996). Two dominant genes that conferred resistance on root-knot nematode, *Meloidogyne arenaria* race 1, were mapped using RAPD and sequence characterized amplified region (SCAR) markers (Garcia et al. 1996). A marker Z3/265, closely linked with nematode resistance, was mapped to a linkage group in a backcross population known to contain *A. cardenasii* introgression (Garcia et al. 1996), which they cloned to make SCAR and RFLP probes that further confirmed the linkage with nematode resistance. The RFLP markers linked to a locus for resistance to *M. arenaria* race 1 has been identified by various workers using mapping populations derived from interspecific crosses (Choi et al. 1999; Church et al. 2000; Seib et al. 2003), thus providing a useful selection method for identifying resistance to the peanut root-knot nematode.

1.7 Approaches to Interspecific Gene Transfer

The differences in ploidy levels have been the major bottleneck in interspecific gene transfer between diploid wild *Arachis* species and tetraploid *A. hypogaea*. For the successful utilization of wild *Arachis* species in the genetic amelioration of the cultivated groundnut cultivars, Simpson (2001) has outlined the following approaches to overcome the genomic imbalances in crosses involving species with different ploidy levels.

1.7.1 Hexaploid Route

A. hypogaea ($2n = 4x = 40$) is hybridized with a diploid wild *Arachis* species ($2n = 2x = 20$) to produce a sterile triploid ($2n = 3x = 30$), which is then treated with colchicine to produce hexaploid ($2n = 6x = 60$). This amphiploid is first crossed and then selfed or backcrossed with *A. hypogaea* until the tetraploid hybrid is obtained after eliminating the excess chromosomes during segregation. This pathway has been used with some success in North Carolina State University, Raleigh, USA, and at ICRISAT for developing numerous disease- and insect-resistant elite germplasm. This technique could be used with several variations such as crossing two or more diploid species before crossing with *A. hypogaea*. This approach has limitation, as it is time consuming and unpredictable. However, the advantage is through selfing as selfing the amphiploids increases recombination between the chromosomes of different genomes.

1.7.2 Diploid/Tetraploid Pathway using Bridge Species

This pathway has been the most successful introgression pathway at the Texas A&M University, USA, for gene transfer from wild *Arachis* species into *A. hypogaea* (Simpson 1991; Simpson and Starr 2001), using B-genome species as a bridge species. *A. cardenasii* was first crossed with *A. diogoi*, both diploid species and the resulting hybrid (52% pollen stained) was crossed as male parent with *A. batizocoi*, the "B"-genome diploid species. The resulting diploid three-way hybrid was sterile (pollen stained <1%) and was subsequently treated with colchicines. The amphiploid (> 90% pollen stained) was easily crossed with *A. hypogaea* cv. Florunner. Selection was made for highly fertile-resistant progenies that were backcrossed to *A. hypogaea*. This approach had been proposed by Smartt et al. (1978b) as a solution to overcome the sterility barrier between *A. hypogaea* and the wild diploid species. They hypothesized that use of a B-genome parent might make the complex amphiploids more cross-compatible with *A. hypogaea*, but the B-genome species, *A. batizocoi*, is susceptible to late leaf spot and other diseases, which may lead to the incorporation of these unfavorable traits into the breeding lines, thus hampering the groundnut improvement programs (Holbrook and Stalker 2003).

1.7.3 Diploid/Tetraploid Pathway

The two diploid wild *Arachis* species are first doubled with colchicines, followed by the hybridization of these two amphidiploids to form a tetraploid hybrid, which is finally crossed with *A. hypogaea*, provided the amphiploid hybrid is fertile enough to make the cross. In order to transfer rust resistance from the wild *Arachis* species, autotetraploidy was induced in three diploid species, viz., *A. cardenasii*, *A. stenosperma* and *A. chacoense*, and the resultant autotetraploids were crossed with *A. hypogaea* cultivars. A number of *A. hypogaea*-like derivatives were identified with rust resistance transferred from wild species. Germplasm lines have not been released from this pathway in peanut till date. High level of sterility is the major factor that limits this technique.

Another variant of this pathway is to first cross two diploid *Arachis* species, double the chromosome number of the hybrid, and then cross the resultant amphidiploid with *A. hypogaea*. This pathway was attempted in Texas (Simpson 1991), but without both A- and B-genome types in the crossing scheme, the success is limited greatly because of high sterility factors.

1.8 Elite Germplasm Originating from Interspecific Crosses

To date, only species from primary and secondary gene pools have been exploited, leading to the development of many elite germplasm lines that originate from interspecific crosses, with resistance to rust, ELS, LLS, nematodes, southern corn rootworm, corn earworm, *Spodoptera*, and leaf hoppers were reported from interspecific crosses [reviewed in Dwivedi et al. (2003)]. However, these elite germplasm are good sources of resistance to many pests and diseases to enhance the levels of resistance in cultivated groundnut.

Spancross (Hammons 1970), Tamnut 74 (Simpson and Smith 1975), Coan (Simpson and Starr 2001), NemaTAM (Simpson et al. 2003), ICGV-SM 85048 (Nigam et al. 1998) and ICGV-SM86715 (Moss et al.

1998) were released for cultivation, mostly in the USA. Nematode resistance has helped US peanut growers to save US$100 million annually (*www.unep.org/documents/default.asp?documentID=399&article*). Likewise, the researchers at ICRISAT were able to improve the levels of resistance to rust and late leaf spot in newly developed breeding lines originating from crosses involving interspecific derivatives in the breeding program. Following interspecific hybridization, 16 breeding lines (ICGV 99001 to ICGV 99016) have been developed at ICRISAT, of which ICGV 99001 and 99004 are resistant to late leaf spot (LLS) and ICGV 99003 and 99005 are rust resistant (Singh et al. 2003).

1.9 New Approach to Interspecific Gene Transfer

Several attempts have been made to transfer variability from wild *Arachis* species into *A. hypogaea* using the methods described in Sect. 1.10. However, limited success has been realized from these approaches. Synthetic amphiploids have proved successful in generating new diversity in crops such as wheat and Brassicaceae [reviewed in Dwivedi et al. (2008)]. Using this approach, Simpson et al. (1993) crossed an AA-genome donor hybrid (*A. cardenasii* × *A. diogoi*) with a BB-genome species, *A. batizocoi*, and treated the resultant sterile hybrid with colchicine to double the chromosome number to obtain fertile hexaploid. This synthetic amphiploid, named TxAG-6, was subsequently crossed and backcrossed with the cultivated groundnut, which resulted in the release of two groundnut cultivars (Coan and NemaTAM) carrying genes for root-knot nematode (*M. arenaria*) resistance from *A. cardenasii* (Simpson and Starr 2001; Simpson et al. 2003). Another germplasm line, TxAG-7, was derived by crossing TP-129 with UF 439-16-10-3, a component line of Florunner (Norden et al. 1969). The four-species F_1 complex hybrid was then backcrossed to UF 439-16-10-3 as female, producing a population of BC_1F_1 plants, one of which was designated TP-135-4, and named TxAG-7 (Simpson et al. 1993). Both the lines TxAG-6 and TxAG-7 carry genes for nematode resistance (Nelson et al. 1989; Starr et al. 1990). However, none of the parental genotypes involved in synthesizing the TxAG-6 are ancestors of cultivated groundnut, not the true synthesis of cultivated groundnut. More recently, the amphiploid synthesized by involving progenitor species, *A. duranensis* and *A. ipaënsis*, produced fertile progenies when crossed with *A. hypogaea* (Favero et al. 2006). Work on synthesizing the amphiploids involving wild species, and their subsequent utilization for the genetic amelioration of the cultivated groundnut is in progress at ICRISAT. The synthetic amphidiploids (tetraploid) have been generated from the diploid hybrids involving AB-genome (*A. duranensis* × *A. ipaënsis*, *A. duranensis* × *A. batizocoi*, *A. duranensis* × *A. hoehnei*, *A. valida* × *A. duranensis*, *A.ipaënsis* × *A. duranensis*, *A. batizocoi* × *A. duranensis*, *A. valida* × *A. duranensis*, *A. kempff-mercadoi* × *A. hoehnei*, *A. batizocoi* × *A. cardenasii*, *A. valida* × *A. diogoi*, *A. magna* × *A. batizocoi*, *A. batizocoi* × *A. cardenasii*), AA-genome (*A. kempff-mercadoi* × *A. stenosperma*, *A. duranensis* × *A. cardenasii*) and BB-genome (*A. trinitensis* × *A. hoehnei*, *A. magna* × *A. valida*), which are being further crossed with cultigens to introduce genes of interest into improved genetic backgrounds using marker-assisted introgression to minimize the linkage drag. This "resynthesizing pathway" would allow the breeders to capture the enormous variability available in the wild species by incorporating various exotic genomes in the synthetic amphidiploids, and its subsequent utilization would help in incorporating the traits of interest from various wild species into the cultivated groundnut background [reviewed in Dwivedi et al. (2008)].

Wild *Arachis* species have many undesirable traits linked with resistance traits: thick shell, highly reticulated, constricted, prominently ridged and conspicuously beaked pods, which are small and catenated. Using conventional crossing and selection, it has been difficult to break such undesirable association due to linkage drag while selecting the progenies from such interspecific crosses. However, with the recent developments in marker technology (both in terms of marker developments, SSRs and DArT, and high throughput assay, ABI3700), it should now be possible to minimize the linkage drag, and monitor and fix the allelic variations associated with beneficial traits in progenies from interspecific crosses.

1.10 Outlook

Genetic variability is the key to the success of crop improvement programs. Plant breeders preferably exploit variation from the primary gene pool of a specific crop. Cultivated groundnut has narrow genetic base, probably because of the bottlenecks associated with its origin. Moreover, for some stresses, the resistance is either not available or present in very low levels in *A. hypogaea*, in spite of the fact that over 14,000 germplasm accessions are locked in national and international genebanks. Wild *Arachis*, in contrast, show enormous genetic variation in the traits most important for the enhancement of groundnut productivity. However, most of these variations detected in secondary, tertiary and fourth gene pools require use of techniques such as ploidy manipulation, bridge crosses and ovule/embryo culture. Using these techniques alone or in combinations, researchers have been able to transfer beneficial traits (mostly resistance to pests and diseases) from secondary gene pool to cultivated groundnut. Several elite germplasm from such interspecific crosses that are resistant to pests and diseases have been released worldwide, of which a few have been released as cultivars. Prominent among these are the two root-knot nematode-resistant (carrying gene from wild relatives) groundnut cultivars in the USA. Likewise, some wild *Arachis* species from the tertiary gene pool have been successfully crossed with *A. Hypogaea*; however, the utility of such crosses towards releasing the genetic variation that is useful for selection is yet to be demonstrated. More recently, tetraploid amphiploids, involving several *Arachis* species, including *A. hypogaea* progenitors *A. duranensis* and *A. ipaënsis*, have been produced. These amphiploids are being further crossed with *A. hypogaea* to unlock the genetic variation from *Arachis* species. Over the past few years, molecular biology research in groundnut has made considerable progress towards developing markers (SSR and DArT) and genetic maps. Today, we have large number of SSR markers, the DArT markers being discovered, the high-throughput assay platform (ABI3700), the AA- and BB-genome-based genetic maps involving wild relatives, tetraploid genetic map for *A. hypogaea* (see Sect. 1.6). It is expected that with the availability of these genomic resources, it should be feasible to minimize the linkage drag when selecting progenies with beneficial traits from interspecific crosses. Several projects are underway to exploit these genetic and genomic resources to broaden the genetic base of *A. hypogaea* germplasm for the development of high yielding groundnut cultivars with specific attributes for the benefit of the farming community globally.

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