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Abstract

Groundnut, a crop rich in nutrients, originated in South America and spread to the rest of the world. Cultivated groundnut contains a fraction of the genetic diversity present in their closely related wild relatives, which is not more than 13 %, due to domestication bottleneck. Closely related ones are placed in section *Arachis*, which have not been extensively utilized until now due to ploidy differences between the cultivated and wild relatives. In order to overcome *Arachis* species utilization bottleneck, a large number of tetraploid synthetics were developed at the Legume Cell Biology Unit of Grain Legumes Program, ICRISAT, India. Evaluation of synthetics for some of the constraints showed that these were good sources of multiple disease and pest resistances. Some of the synthetics were utilized by developing ABQTL mapping populations, which were screened for some biotic and abiotic constraints. Phenotyping experiments showed ABQTL progeny lines with traits of interest necessary for the improvement of groundnut.

8.1 Introduction

Groundnuts (*Arachis hypogaea* L.) are rich in nutrients, providing over 30 essential nutrients and phytochemicals. They are a good source of niacin

(Whitley et al. 2011), which plays a role in brain health and blood flow, folate, fiber, magnesium, vitamin E, manganese, and phosphorus (Savage and Keenan 1994). Plumpy'Nut, a ready-to-use therapeutic food made from groundnut, is a popular source of nutrient used by UNESCO to treat acute malnourished kids in Africa. Groundnuts contain about 25 % protein, a higher proportion than in any true nut. Recent research on groundnuts and nuts in general has found antioxidants (Yu et al. 2005) and other chemicals, which may provide health benefits. Roasted groundnuts rival the antioxidant content of blackberries and strawberries and are far richer in antioxidants than carrots or beets.

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Research shows that groundnuts contain high concentrations of antioxidant polyphenols (Craft et al. 2010). Groundnuts are a significant source of resveratrol, and the amount of resveratrol equivalent to that present in red grapes (Sanders et al. 2000), a chemical associated with reduction in the risk of cardiovascular disease (Fraser et al. 1992; Hu et al. 1998; Prineas et al. 1993) and cancer (Awad et al. 2000) and having antiaging properties, hence would have high impact in both health and cosmetic industries. Groundnuts are a source of coenzyme Q10 (Pravst and Zmitek 2010), as are oily fish, beef, soybeans, and spinach. It is believed that the crop has originated in South America. According to Vavilov (1951), it was first domesticated in the Brazilian-Paraguayan region. The area of the valleys of Paraguay and Parana rivers is the most likely center of origin of this legume. Excavation in coastal Peru dating back to 800 BC shows the cultivation of groundnut. From South America, groundnut spread to other parts of the world. It was commonly found in the West Indies, but not in the United States in pre-Columbian times. Groundnut was introduced to the Old World in the sixteenth century when the Portuguese took the seeds from America to Africa. The Spaniards introduced it into the Philippines. It then spread to China, India, Japan, Malaysia, and other parts of the world.

Human interaction through selection of most suited lines over the centuries resulted in loss of much of the genetic diversity/desirable alleles and genes whose importance is now being realized. Further, we are still unaware of future preference for the so-called “lost” genetic diversity which includes genes for resistance/tolerance to biotic and abiotic stresses as well as taste and nutritional composition along with yield. Although such concerns are raised at majority of the scientific gatherings, still not much initiative has been taken even for very important food crops. Hence, this is the prime time to retrieve desirable alleles not only to address existing problems but also for the future as well in order to sustain food production. Although pre-breeding utilizing wild gene pool does not produce new varieties, it does turn up intermediate products that breeders find easier to use. It throws up enough variation in the crops such as groundnut

to sustain breeding activities especially with the assistance of molecular markers. Many public agricultural research institutions, such as the International Rice Research Institute (IRRI), International Maize and Wheat Improvement Center (CIMMYT), International Institute of Tropical Agriculture (IITA), and International Crops Research Institute for Semi-Arid Tropics (ICRISAT), have active pre-breeding programs in their mandate crops. Pre-breeding is the link between conservation and use of crop wild relatives. Out of all the raw materials at the breeder’s disposal, the diversity of crop wild relatives has been relatively neglected. The conserved germplasm in the gene banks is for the present and future use. With urbanization, explosion in population growth, and dwindling water resources and being in a 2 °C warmer world, we may not have a choice but to bring in new sources of variation through pre-breeding.

8.2 Taxonomy

The cultivated groundnut (*Arachis hypogaea* L.), an annual herb belonging to the family Fabaceae (Leguminosae), is classified into two subspecies, subsp. *fastigiata* Waldron and subsp. *hypogaea* Krap. et. Rig. The subsp. *fastigiata* contains four botanical varieties, var. *vulgaris*, var. *fastigiata*, var. *peruviana*, and var. *aequatoriana*. The subsp. *hypogaea* contains two varieties, var. *hypogaea* and var. *hirsuta*. Each of these botanical types has different plant, pod, and seed characteristics (Krapovickas and Gregory 1994). Groundnut is an allotetraploid ($2n=2x=40$) with “AA” and “BB” genomes. All species, except the cultivated species (*A. hypogaea* and *A. monticola*) in section *Arachis* and certain species in section *Rhizomatosae*, are diploid ($2n=2x=20$). The diploid progenitors, *A. duranensis* and *A. ipaensis*, contributed “AA” and “BB” genomes, respectively, to the cultivated groundnut (Kochert et al. 1996). The phylogenetic analyses based on intron sequences and microsatellite markers also provide evidence for this hypothesis (Moretzsohn et al. 2012). A single hybridization event between the diploid progenitors followed by chromosome

doubling (Kochert et al. 1996) about 3,500 years ago led to the origin of cultivated groundnut. Krapovickas and Gregory (1994) used taxonomy and crossability studies to classify 69 *Arachis* species into nine sections. The additional 11 species described by Valls and Simpson (2005) also come under these nine sections, making the total to 80 *Arachis* species. Sections *Trirectoides*, *Erectoides*, *Extranervosae*, *Triseminatae*, and *Heteranthae* are treated as older sections while *Procumbentes*, *Caulorrhizae*, *Rhizomatosae*, and *Arachis* are of more recent origin.

are the major insect pests in groundnut, causing serious damage (Wightman and Amin 1988). Groundnut borer or weevil (*Caryedon serratus*) and rust-red flour beetle (*Tribolium castaneum*) are the major storage insect pests in groundnut. Nutritional traits which include oil, protein, sugar, iron, and zinc content, fatty acid profile, and freedom from toxins are important. The major issue being the presence of sources of resistance in cultivated germplasm is low to moderate compared to high levels of resistance in wild *Arachis* species.

8.3 Production-Related Problems

There is a large gap between potential pod yield and the realized pod yield in most of the groundnut growing areas (Johansen and Nageswara Rao 1996). Abiotic stresses such as drought and high temperature are important yield-reducing factors. Groundnut is attacked by several fungal pathogens such as late leaf spot (LLS) caused by *Phaeoisariopsis personata* (Berk. & Curt.) von Arx, early leaf spot (ELS) caused by *Cercospora arachidicola* Hori, and rust caused by *Puccinia arachidis* Spegazzini which are among the important foliar fungal diseases worldwide. Aflatoxins are potent carcinogens produced by *Aspergillus* spp. *A. flavus* is the predominant species in Asia and Africa. Stem and pod rot, caused by *Sclerotium rolfsii*, is a potential threat to groundnut. Apart from this, groundnut is prone to several virus diseases such as groundnut rosette disease (GRD), peanut bud necrosis disease (PBNB), peanut stripe potyvirus (PStV), peanut stem necrosis disease (PSND), and peanut clump virus disease (PCVD). Bacterial wilt, caused by *Ralstonia solanacearum*, is predominant among bacterial diseases of groundnut. Globally, nematodes cause 11.8 % yield loss in groundnut (Sharma and McDonald 1990). Aphids (*Aphis craccivora* Koch), several species of thrips (*Frankliniella schultzei*, *Thrips palmi*, and *F. fusca*), leaf miner (*Approaerema modicella*), red hairy caterpillar (*Amsacta albistriga*), jassids (*Empoasca kerri* and *E. fabae*), and *Spodoptera*

8.4 Evolution and Diversity in Cultivated Groundnut

Cultivated groundnut contains a fraction of the genetic diversity which is not more than 13 % (Varshney et al. 2009), found in their closest wild relatives in section *Arachis* (Kochert et al. 1991), a legacy of the “domestication bottleneck.” Groundnut has an interesting evolutionary history. The domesticated groundnut is an amphidiploid or allotetraploid, meaning that it has two sets of chromosomes from two different species, thought to be *A. duranensis* and *A. ipaensis* (Kochert et al. 1991, 1996; Seijo et al. 2007). These species combined in the wild to form the tetraploid species which gave rise to the domesticated groundnut. The first domestication bottleneck was the combination of two species *A. duranensis* and *A. ipaensis* among 26 species from section *Arachis*. Crossing experiments between *A. duranensis* and *A. ipaensis* have shown that the diploid hybrid is highly sterile (Mallikarjuna et al. 2011a). Had the hybrid been fertile, there would not have been the need to double its chromosome number to form the fertile amphidiploid. It would probably have remained a diploid than a tetraploid as it is today. So the second bottleneck was in the formation of a diploid sterile hybrid. The third bottleneck was in process of chromosome duplication to form the allotetraploid as it is known in literature that polyploidy causes genetic bottlenecks (Sanford 1983). Ancient farmers would have selected relatively few plants from the progenitors of

modern crops in a limited number of places, and a similar situation would have existed for groundnut in South America. This can be visualized as the fourth bottleneck. The early Portuguese and Spanish traders during their expeditions spread the crop to the rest of the world thus giving rise to yet another, the fifth, bottleneck, superimposed by the sixth bottleneck which is the self-pollinating nature of the crop. To conclude, groundnut is the product of evolution after a series of six bottlenecks.

8.5 Utilization of Wild Relatives

Arachis species from section *Arachis*, which are true diploid with $2n=20$, have been extensively utilized in crosses with cultivated groundnut ($2n=40$) utilizing various pathways of introgression (Simpson and Starr 2001), and traits of interest such as resistance to nematode, late leaf spot, rust, and *Spodoptera litura* transferred (Garcia et al. 1996; Burrow et al. 1996; Singh et al. 2003; Mallikarjuna et al. 2004a, b). GPBD4, a groundnut genotype resistant to rust and LLS, was released for commercial cultivation in India (Gowda et al. 2002). One of its parents, ICGV 86855, is an interspecific derivative between *A. hypogaea* and *A. cardenasii*, resistant to rust and LLS, and was developed at ICRISAT, India. Members of section *Procumbentes* have been successfully crossed and fertile progeny has been obtained using in vitro techniques. Progeny lines were screened for various traits of interest. Another member of section *Procumbentes*, *A. kretschmeri*, was also crossed successfully (Mallikarjuna and Hoisington 2009). *A. chiquitana* that was previously placed in section *Procumbentes* (Krapovickas and Gregory 1994) has now been moved to section *Arachis* (Robledo et al. 2009). F_2 seeds were screened for *A. flavus* infection, and many of the seeds did not have any *A. flavus* infection, whereas the control and some of the other F_2 seeds had *A. flavus* infection (Mallikarjuna 2005). *A. glabrata* belonging to section *Rhizomatosae* was successfully crossed with *A. duranensis* and *A. diogeni* (Mallikarjuna 2002) and *A. hypogaea* (Mallikarjuna and Sastri 2002) using embryo

rescue techniques (Mallikarjuna and Sastri 1985). The interest at present is to exploit the genetic diversity present in section *Arachis* through the development of tetraploid amphidiploid and autotetraploid groundnuts which are also called synthetic groundnuts.

Inadequate levels of resistance in peanut germplasm are one of the important factors for not having resistance to *A. flavus* aflatoxin in peanut. This means sources of resistances have to be scouted beyond the cultivated primary gene pool. The report from Xue et al. (2005) showed that *Arachis* species *A. duranensis* (8 accessions) and *A. cardenasii* (2 accessions) from section *Arachis* had high levels of resistance to aflatoxin production and interspecific derivatives obtained from them continued to show the trait. ICRISAT screened advance generation lines derived from *A. cardenasii* (10,017 lines) in aflatoxin sick plot for three consecutive years and found many of the lines with low aflatoxin production. This opens up new avenues for aflatoxin resistance breeding in peanut (Mallikarjuna N and Sudini H, unpublished data). Sources of resistance to late leaf spot caused by *Cercosporidium personatum* (Berk. & M.A. Curtis) are higher in wild *Arachis* species (Subrahmanyam et al. 1985b) as compared to moderate levels of resistance in cultivated germplasm (Dwivedi et al. 2002). *Arachis cardenasii*-derived lines showed resistance to LLS, when screened under unprotected field conditions in different locations (Mallikarjuna N and Sudini H, unpublished data). Peanut bud necrosis disease (PBND) is an economically important virus disease of peanut in many Asian countries where peanut is grown. The disease causes crop losses exceeding 89 million US dollars in India alone (Anon 1992). Sources of resistance are absent in cultivated germplasm (Reddy 1998). Many of the *Arachis* species have been found to be resistant to the disease (Reddy et al. 2000). Stable lines derived from *Arachis* species were screened for PBND under disease hot spot locations, and a few resistant lines were identified (Sunkad G and Mallikarjuna N, unpublished data).

Among the soilborne fungal diseases of peanut, stem rot caused by *S. rolfisii* is a potential threat to peanut production throughout the world.

The disease causes severe damage during any stage of crop growth, and yield losses over 25 % have been reported by Mayee and Datar (1988). Sources of resistance to the constraint is not up to the desired level in cultivated gene pool. Stable lines derived from *Arachis* species were screened for *S. rolfisii* in the disease hot spot location at Dharwad, Karnataka State, India, and a few lines with durable resistance were obtained (Kenchana Gowdar and Mallikarjuna N, unpublished data). *Spodoptera litura*, also called fall armyworm, a polyphagous insect, is becoming an important insect pest of groundnut with sources of resistance to the pest absent in the cultivated gene pool. Yield losses of peanuts have been directly associated with higher density of larvae of *S. litura* and the intensity of defoliation (Panchbhavi and Nethradani 1987). Currently, no cultivars of peanut are known to express reasonable resistance to *S. litura*. However, some wild relatives of peanut were found resistant to *S. litura*. Neonate larvae suffer high levels of mortality, and the development of older larvae on resistant wild species is severely inhibited (Stevenson et al. 1993b). Stevenson et al. (1993a) identified flavonoids chlorogenic acid, quercetin, and rutin present in *Arachis kempff-mercadoidi* responsible for resistance to *S. litura*. Mallikarjuna et al. (2004a) developed lines utilizing *A. kempff-mercadoidi* and screened lines for *S. litura* resistance. Resistant derivatives were found to have high levels of flavonoids, and anti-biosis mechanism prevented larval growth. Susceptible derivatives and the female parent *A. hypogaea* had low levels of flavonoids (Mallikarjuna et al. 2004b).

8.6 Development of Synthetics for Groundnut Improvement

Tremendous progress has been made in wheat and brassica, two similar polyploid genera in the development and utilization of synthetics, by combining the putative genome donors of the cultivated species. This triggered the development of tetraploid amphidiploids (synthetics) in groundnut. Until recently, there were three sources of

amphidiploids or new sources of tetraploid *A. hypogaea* available in public domain. The first one originated from a cross between *A. cardenasii* Krapov. et W.C. Gregory, *A. diogoi* Hoehne, and *A. batizocoi* Krapov. et W.C. Gregory, and it was utilized to develop backcross progeny lines (Simpson et al. 1993). Two groundnut cultivars, namely, COAN (Simpson and Starr 2001) and NemaTAM, were released utilizing this source. More recently, an amphidiploid was constructed utilizing *A. ipaensis* and *A. duranensis* (Favero et al. 2006). This amphidiploid is being used by Brazil and Senegal to develop backcross population and chromosome substitution lines. Preliminary mapping data indicated low level of marker segregation distortion in F₂ population utilizing amphidiploid (Dwivedi et al. 2008). A successful effort for genome-wide segment introgressions from a synthetic amphidiploid (*A. ipaensis* × *A. duranensis*) to a cultivated variety (Fluer 11) using molecular markers has already been reported (Foncéca et al. 2009). The third one is a cross between *A. gregoryi* and *A. linearifolium* (GCP 2005), and there is no information of using this source for peanut improvement. With this background, the Legume Cell Biology Unit of ICRISAT under the stewardship of N. Mallikarjuna has generated many new sources of tetraploid groundnut utilizing many more *Arachis* A, B, and K genome species, not used until now to generate new sources of tetraploid/synthetic groundnut (Mallikarjuna et al. 2011a). Traditionally, wild relatives of peanut were directly used in crossing program producing triploids as *Arachis* species in the compatible gene pool are diploids and cultivated groundnut is a tetraploid. Triploids are cumbersome to use for groundnut improvement, as they demand an elaborate backcross program, but such efforts have not gone without dividends (Mallikarjuna et al. 2004a, b). Among the published 17 new sources of synthetics (Mallikarjuna et al. 2011a), one (ISATGR 1212) had putative genome donors and it comprised of *A. duranensis* and *A. ipaensis*, and another one (ISATGR 40A) had the reciprocal combination (*A. ipaensis* × *A. duranensis*). Three of them had at least one of the genome donors of *A. hypogaea*, i.e., either *A. duranensis* or

A. ipaensis. Since there is only one accession of *A. ipaensis* reported to date, it can be presumed that it is indeed the B genome donor. With respect to *A. duranensis*, there are many accessions available in different gene banks across the world, and molecular analysis has shown that there are some differences between the accessions (Husain and Mallikarjuna 2012) as well as with respect to traits. *A. duranensis* accession ICG 8139 is of the earliest flowering accessions in the ICRISAT gene bank (Mallikarjuna N, unpublished data). Five amphidiploids had K genome either with A or B genome; hence these would be totally new combinations available for the improvement of groundnut. Among the five autotetraploids synthesized with A genome species, there is diversity with respect to the groups they belong, for example, one autotetraploid ISATGR 90B comprised of two A genome species *A. kempff-mercadoi* and *A. stenosperma*. *A. kempff-mercadoi* belongs to group Chiquitano. Members of the group Chiquitano grow in the southern and western portion of the Chiquitania biogeographic region of the Santa Cruz Department of Bolivia (Robledo et al. 2009). Another autotetraploid ISATGR 99B is made up of *A. diogoi* and *A. cardenasii*. *A. diogoi* belongs to the Pantanal group and *A. cardenasii* belongs to Chiquitano group. Robledo et al. (2009) have shown variability in heterochromatin and 18S–26S rRNA loci between the members belonging to Chiquitano and Pantanal groups. Hence, the autotetraploids generated are also important sources of variation which can be exploited for broadening the genetic base of cultivated groundnut.

Apart from the published new sources of synthetics, a few more synthetics have been generated since then (Mallikarjuna N, unpublished data). They include ISATGR 47A comprising of *A. valida* × *A. duranensis*. Here, the accession of *A. valida* was different than that of ISATGR 168B reported by Mallikarjuna et al. (2011a). ISATGR 72B is made up of *A. duranensis* and *A. cardenasii*. ISATGR 163B is made up *A. kempff-mercadoi* and *A. stenosperma*. A different accession of *A. kempff-mercadoi* was used in this cross than the one used to develop ISATGR 80A (Mallikarjuna et al. 2011a). In attempts to broaden the genetic

base and introduce variation into cultivated groundnut, double synthetics were generated by combining the genomes of two synthetics. Care was taken to see that at least three *Arachis* species, and a maximum of four, contributed their genomes. There were five such sources developed, ISATGR 1212 (*A. duranensis* × *A. ipaensis*) X ISATGR 9A (*A. batizocoi* × *A. cardenasii*), ISATGR 1212 (*A. duranensis* × *A. ipaensis*) X ISATGR 5B (*A. magna* × *A. batizocoi*), ISATGR 278-18 (*A. duranensis* × *A. batizocoi*) X ISATGR 5B (*A. magna* × *A. batizocoi*), and ISATGR 1212 (*A. duranensis* × *A. ipaensis*) X ISATGR 265-5A (*A. kempff-mercadoi* and *A. hoehnei*) (Shilpa et al. 2013), and it was observed that although synthetics selected to develop double synthetics were stable and fertile, not all double synthetics were fertile. One of them [ISATGR 278-18 (*A. duranensis* × *A. batizocoi*) X ISATGR 11A (*A. magna* × *A. valida*)] did not set any pods/seeds, hence was a genetic dead end. The rest of the double synthetics set pods/seeds and hence were considered valuable sources of variation. All the synthetics and double synthetics produced single-seeded pods being larger in size in majority of the cases but resembling the wild species pods in shape and reticulation. An interesting feature was observed in the pod traits from double synthetic ISATGR 278-18 (*A. duranensis* × *A. batizocoi*) X ISATGR 5B (*A. magna* × *A. batizocoi*). Many of the pods were double seeded which did not have peg, as seen in many wild *Arachis* pods, and resembled those of *A. hypogaea* pod shape and pod wall architecture. Such a feature was not observed in any *Arachis* species conserved in the ICRISAT gene bank, or in the combination of *Arachis* species during the formation of synthetics, or in any other double synthetics generated in this study. Double-seeded pods from this cross may be a case of evolution fast-forward, as they resemble those of *A. hypogaea*. It will be interesting to see if only double-seeded pods can be recovered from this cross a few generations later.

Cultivated groundnut is susceptible to late leaf spot (LLS) caused by *Phaeoisariopsis personata* [(Berk. & M.A. Curtis) Aex], and the resistance is low to moderate in the primary gene pool of groundnuts (Dwivedi et al. 2002). Closely related

wild species in the secondary gene pool are highly resistant to the disease (Subrahmanyam et al. 1985a). Diploid *Arachis* species are difficult to use for the introgression of LLS resistance due to ploidy differences between cultivated and *Arachis* species. Synthetics were screened for LLS in a disease hot spot location at Raichur. All the tetraploids, except for ISATGR 155, showed resistance to the disease (Shilpa et al. 2013). Utilizing one of the synthetics, namely, ISATGR 265-5, and crossing it with cultivated groundnut yielded BC₂F₃ lines. These lines were screened for LLS. Screening results showed that some of the progenies had LLS resistance (Sudini H and Mallikarjuna N, unpublished data), thus showing that synthesized tetraploids are good sources of LLS resistance. Mallikarjuna et al. (2012a) studied the components of LLS resistance such as incubation period, leaf area damage, lesion number and diameter, latent period of infection, and infection frequency in some of the synthetics using detached leaf technique. The studies gave a clearer picture of LLS resistance in the synthetics. Synthetics were screened for the presence of resveratrol, and its presence was observed in the few lines (Padmashree and Mallikarjuna, unpublished data). Peanut bud necrosis disease is an economically important disease causing yield losses up to 89 million USD in India alone (Anon 1992), and the sources of high levels of resistance are lacking in the cultivated germplasm, but many wild *Arachis* species have reasonable resistance (Reddy et al. 2000). Some of the synthetics were screened for PBNB in a disease hot spot at Raichur, India, and many of them were found to have immune to resistant reaction to the disease (Shilpa et al. 2013). Since tetraploids can be easily crossed with cultivated peanut, sources of resistance in tetraploids open up new opportunities to breed PBNB-resistant groundnuts. Plant proteinase inhibitors are known for improving defense against insects and pathogens (Ryan 1990). Trypsin and chymotrypsin inhibitors have been described as potential cancer or chemo-protective agents (Clemente et al. 2005). Many of the synthetics used in the study by Shilpa et al. (2013) showed high PI activity compared to that present in the cultivated groundnut varieties.

This may be one of the reasons for the presence of disease and pest resistance in the synthetics. PIs are known to prevent the target insect from digesting protein by competitively binding to the active site of proteinase, which is the actual binding site of proteinase. As the insect cannot digest protein, it is subjected to starvation and/or death. PIs are also known to cause increased levels of insect deformity, due to the potential inhibition of the proteinases involved in the metamorphosis of the larvae (Prasad et al. 2010). Screening the synthetics for the presence of proteinase inhibitory activity against bovine pancreatic trypsin and chymotrypsin showed activity against midgut trypsin-like proteinases of *Spodoptera litura* (Padmashree and Swathi unpublished data). A new role for PI in the modulation of apoptosis or programmed cell death has been identified in soybean (Koslak et al. 1997). Although PI was also present in the cultivated groundnut, S. Marri and K. Padmashree (unpublished data) observed that the molecular components of those present in the cultivated and the synthesized tetraploids were different, and these components have a differential role with respect to insect midgut trypsin-like proteinases of *Spodoptera litura*. Nutritional composition which is composed of oil, fatty acid composition, O/L ratio (oleic to linoleic fatty acid ratio), amount of protein, and iodine value were studied (Shilpa et al. 2013). O/L ratio is an important factor in deciding the stability of the oil. Fatty acid composition is made up of unsaturated fatty acids (TUSF) and saturated fatty acids (TSF), which make up the physical and chemical properties of the oil. Variation was observed for total protein concentration. In the cultivated peanut accessions, total protein concentration ranged from 65 to 85 mg/g compared to a range of protein concentration of 43–134 mg/g in the synthetics. High-protein concentration synthetics can be selected to breed for high-protein groundnut lines in those regions of the world, where groundnut is not only an oilseed crop but is used as a food crop too. Some difference was observed with respect to percent oil content between the cultivated groundnut varieties (approx. 40 %) and synthetics (45–57 %) (Shilpa et al. 2013). With respect to O/L ratio, it

was 1.0 in cultivated varieties, and in the synthetics, it varied from 0.8 to 1.3 (Shilpa et al. 2013). Not much difference was observed between the cultivated varieties and synthetics with respect to fatty acid composition (excluding oleic and linoleic). Iodine content (IV), a measure of the degree of unsaturation which has been commonly used as an indicator of predicting shelf life (Mercer et al. 1990), was 88–105 in synthetics compared to 90–95 in groundnut varieties.

In order to study meiotic recombinations between the cultivated groundnut and the newly developed synthetics, a range of synthetics were crossed with a few cultivars of *A. hypogaea* (Mallikarjuna et al. 2012b). The study showed good recombination between cultivated and synthetics with high pollen fertility in the hybrids, thus showing that synthetics form a good source of variation, which can be successfully utilized for broadening the genetic base of the cultigen. More recently, double synthetics were used in the crossing program, and a high level of meiotic recombination was observed (Mallikarjuna N, unpublished data). Fonceka et al. (2012) used a synthetic amphidiploid (Favero et al. 2006) and applied a conventional breeding scheme to capture the genetic diversity in peanut wild relatives. In their study, a set of 122 introgression lines (IL) that offered an extensive coverage of the cultivated peanut genome with generally a unique fragment per line and overlapping fragments between contiguous lines were developed. Their findings opened new avenues for peanut improvement using new sources of tetraploid/synthetic groundnuts. Realizing the scope for advanced backcross (ABQTL) breeding in the improvement of groundnut, initiatives have been taken at ICRISAT to develop three ABQTL mapping populations utilizing three sources of synthetics. These populations were segregated for several biotic, abiotic, and agronomic traits. A subset of BC2F1 individuals was genotyped with DArT markers to construct genetic maps. Advance generation lines (BC2F3) from all populations were screened for a range of biotic and abiotic traits such as O/L ratio, LLS, rust, PBNB, and other yield-related traits. These initial screening experiments revealed a range of useful traits present in

the populations. Population one was developed by utilizing synthetic amphidiploid ISATGR 1212 (Mallikarjuna et al. 2011a), which is composed of putative genome donors of *A. hypogaea* (*A. duranensis* × *A. ipaensis*). Screening the synthetic ISATGR 1212 for LLS and rust showed that it is highly resistant to LLS and rust (Mallikarjuna et al. 2012b). In population one (pop I), 16 % of the lines had moderate levels of resistance to LLS (score of 4, on a scale of 1–9). Majority of the lines had a score of 2 for rust (on a scale of 1–9). O/L ratio in the population varied from 1 to 4 with 50 % of the lines having a ratio of 2–3 compared to a ratio of 1 in cultivated lines used in the study. Only one line had a ratio of 4. The population was screened for peanut bud necrosis disease (PBNB) in Raichur, which is a disease hot spot in the Karnataka State of India. The cultivated check line showed 48 % disease incidence. Two lines were devoid of the disease. Many (40 %) of the test lines from pop I had 20 % or less disease incidence, and 5 % of the lines had 10 % or less disease damage. Stem rot of groundnut caused by *Sclerotium rolfsii* is an economically important disease with sources of resistance lacking in the cultivated gene pool. Pop I (97 lines) was screened for the disease in a hot spot location, and 36 lines (37 % of the screened lines) did not show any disease symptoms. Hence, these can be classified as immune to the disease. The progenies were evaluated in a replicated trail along with the recurrent parent and some popular checks for dry pod yield and other yield parameters. The dry pod yield per plant of the progenies was between 3 and 17 g per plant higher compared to the recurrent parent ICGV 91114 that produced about 13 g per plant. Similarly, shelling outturn (ranged from 54 to 74 %) and 100-seed weight (HSW) (23 to 42 g) were higher than the recurrent parent mean values in the trial (63 % shelling outturn and 32 g of HSW). The proportion of sound mature kernels (SMK) in the progenies ranged from 75 to 97 % that is slightly higher than ICGV 91114 (93 %). Population 2 (pop II) was developed utilizing synthetic amphidiploid ISATGR 265-5 (*A. kempff-mercadoi* × *A. hoehnei*), which does not have any putative genome donors of *A. hypogaea*

(Mallikarjuna et al. 2011a). Population two had better levels of resistance to LLS compared to pop I. Forty percent of the lines had a score of 3 for LLS, and 58 % of the lines had a score of 4 (on a scale of 1–9). Most of the lines had a score 2 for rust (on a scale of 1–9). O/L ratio in the lines was higher in pop II compared to pop I. In four lines, the ratio was 4 or above but less than 5. In 41 % percent of lines, the ratio was above 2 and 57 % percent of lines had a ratio of more than 3. Thirty-eight percent of lines from pop II did not show any *S. rolfisii* symptoms, when screened for the disease in the hot spot location. These lines can be classified as immune to the disease. The dry pod yield in the progenies ranged between 4 and 27 g, which was lower as compared to the recurrent parent ICGV 87846, which had a mean of 36 g. Nevertheless, the other yield parameters of the progenies were slightly better than the mean value of ICGV 87846. The SMK of progenies ranged from 58 to 95 %, shelling outturn ranged from 55 to 74 %, and HSW ranged from 26 to 51 g, as compared to the recurrent parent ICGV 87846 (SMK 86 %, shelling outturn 68 %, and HSW of 44 g).

Breeders often encounter comprised yield and/or pod and kernel features when they use wild species in groundnut breeding programs. However, the results from the evaluation studies of ABQTL populations showed that it is possible to circumvent this constraint. It is possible to combine high levels of resistance from the wild species with high yield potential of elite breeding lines as seen in the progenies. The progenies having high levels of resistance as well as desirable yield levels can be carried forward to the advance generations for use in groundnut breeding as potential sources of variability or breeding lines. Nevertheless, to draw valid conclusions, a more thorough evaluation in fixed lines (of advance generations) may be desirable given the quantitative nature of the inheritance of yield and other yield parameters.

Population 3 (pop III) was derived utilizing synthetic ISATGR 278-18 (*A. duranensis* × *A. batizocoi*) (Mallikarjuna et al. 2011a). *A. duranensis* is one of the genome donors of *A. hypogaea*, but *A. batizocoi* is not. Two lines had a LLS dis-

ease score of 2 and majority of the lines had a score of 3. All the lines had a score of 2 for rust. The population was studied for the presence of proteinase inhibitors. Many of the lines showed maximum TI activity of 8–9 TI units/mg protein, and some lines had a minimum of 2–3 TI units/mg proteins, and in CI activity maximum was 3 CI units/mg protein, whereas in the cultivated species, maximum TI activity was 2–3 TI units/mg protein and CI activity maximum was 2 CI units/mg protein. To examine the specificity of inhibitors on *Spodoptera litura* larvae feeding on groundnut, the gut extracts were also assayed for inhibition of trypsin and chymotrypsin specificities. Maximum of 2 gut units were observed in pop III, and 1 gut unit was observed in cultivated species. The presence of PI in pop III shows that they may play a major role in pest resistance (Shilpa and Mallikarjuna unpublished data).

8.7 Molecular Markers, Genome Mapping, and Genomics as an Adjunct to Breeding

The first groundnut variety developed through integrated marker-assisted selection (MAS) was NemaTAM, a root-knot nematode-resistant variety (Simpson et al. 2003). Identification of a major QTL (QTL rust 01) contributing up to 82.96 % phenotypic variation for rust resistance, it was introgressed through MABC to improve three popular groundnut varieties (ICGV 91114, JL 24, and TAG 24) for rust resistance using GPBD4 as a donor genotype. Several promising introgression lines with remarkable reduction in disease spread and other desirable agronomic traits have been selected for further multiplication and generation advancement (Pandey et al. 2012). Availability of a large number of markers in recent years has ensured limiting linkage drag through stringent background selection and tracking the presence of non-desirable genomic region from the wild relatives. Several reviews provided in-depth information on development, availability, and deployment of genomic resources in groundnut published most recently (Pandey et al. 2012; Janila et al. 2013) and sug-

gested the use of molecular markers in routine breeding programs. Although simple sequence repeat (SSR) markers still rule the hearts of plant breeders for use in genetics and breeding applications, other genotyping systems such as diversity array technology (DArT) and single nucleotide polymorphisms (SNPs) hold the key role for future groundnut improvement. ICRISAT in collaboration with DArT Pvt. Ltd, Australia, developed DArT arrays with 15,360 features which showed low polymorphism among genotypes of primary gene pool. Despite low polymorphism, these markers are of great help in monitoring the alien genome introgression in the cultivated species as observed in the case of pigeon pea (Mallikarjuna et al. 2011b). Furthermore, realizing the great potential role of SNPs, thousands of SNPs were identified in groundnut by the University of Georgia (8486 SNPs) and the University of California-Davis (>2,000 SNPs). To deploy abovementioned marker resources, a range of cost-effective SNP genotyping platforms have become available such as Illumina GoldenGate assays for genotyping 768 SNPs by the University of California-Davis, USA, and 1536 SNPs for groundnut by the University of Georgia, USA. Similarly, an alternative genotyping assay (KASP assay) developed by LGC Genomics (www.lgcgenomics.com/genotyping/kasp-genotyping-reagents, Semagn et al. 2013) provides flexibility to genotype any number of samples with any number of SNPs. Thus, ICRISAT has developed KASP assays for 90 SNPs in groundnut (Khera et al. 2013).

Breeders have been continuously struggling to handle linkage drag using conventional approaches. On the other side, genomic approaches provided reliable and precise solution for monitoring genome-wide alien introgression in elite lines. Thus, integration of genomic tools with conventional breeding approaches promises to enrich cultivated gene pool which will help in harnessing available rich diversity of wild relatives possessing superior alleles. Several synthetics have been developed so far (Simpson et al. 1993; Favero et al. 2006; Mallikarjuna et al. 2011a), providing opportunity to diversify the primary gene pool and conduct ABQTL analysis.

A subset of the abovementioned two populations (ABQTL pop I and ABQTL pop II) was genotyped with DArT markers to construct genetic maps and conduct ABQTL analysis. Already one study reported genome-wide segment introgressions using markers from a synthetic amphidiploid (*A. duranensis* × *A. ipaensis*) into the genetic background of the cultivated variety (Fluer 11) (Foncéka et al. 2009). Therefore, availability of cost-effective genotyping systems in recent years has accelerated the introgression of useful traits and thus improvement of groundnut.

8.8 Conclusion

There is ample genetic diversity in the wild gene pool which harbors several useful genes for groundnut improvement. Direct utilization of diploid *Arachis* species, which are closely related, is cumbersome due to ploidy differences between the cultivated and wild *Arachis* species germplasm. Utilization of distantly related *Arachis* species needs in vitro interventions (Mallikarjuna and Sastri 1985), and utilization of both closely and distantly related species in secondary and tertiary gene pools needs an elaborate backcross program for alien introgressions and obtaining stable tetraploid lines. Ample variation is now available in the form of synthetics and double synthetics (Mallikarjuna et al. 2012a, b; Shilpa et al. 2013). Research experience in the utilization of synthetics has shown that stable tetraploid lines with alien introgressions can be achieved in a shorter period of time and the utilization of suitable molecular markers further accelerates the research programs. The best option for alien introgressions in groundnut is through the utilization of already available tetraploid synthetics and double synthetics in the breeding programs. It is also necessary to develop new sources of tetraploid synthetics so that ample variation is available for groundnut improvement. A range of groundnut synthetics (Mallikarjuna et al. 2011a) are available at ICRISAT (Sharma et al. 2013) for groundnut researchers for utilization in their breeding programs for groundnut genetic improvement.

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