Chapter 8

Marker-Assisted Selection for Biotic Stress Resistance in Peanut

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Abstract

Peanut is the second-most important legume grown worldwide. Cultivated peanut is a disomic tetraploid, 2n=4x=40, with limited genetic diversity due to a genetic bottleneck in formation of the polyploid from ancestors *A. duranensis* and *A. ipaënsis*. Consequently, resistance to biotic stresses is limited in the cultigen; however, wild species possess strong resistances. Transfer of these resistances is hindered by differences of ploidy, but production of synthetic amphidiploids, coupled with use of molecular markers, enables efficient gene transfer. Marker maps have been made from interspecific crosses, and SSR-based maps from cultivated parents have been developed recently. At least 410 resistance gene analogues have been identified. The first markers for biotic stress tolerance were for root-knot nematode resistance and introgressed from one *A. cardenasii* chromosome. These and improved markers have been used for marker-assisted backcrossing, contributing to release of three cultivars. Additional QTLs have been identified since. Early and late leafspots cause significant yield losses worldwide, and resistance depends on multiple genes. Using interspecific populations, five resistance QTLs for early leafspot were identified using greenhouse inoculations, and five QTLs for late leafspot were identified using detached leaf assays. Using cultivated species populations, 28 QTLs were identified for LLS resistance; all but one were minor QTLs; the major QTL was donated by an interspecific introgression line parent. Rust often occurs alongside leafspots, and rust resistance was characterized as one major QTL, plus several smaller QTLs. Marker-assisted backcrossing of this major QTL has been performed into different populations. QTLs for resistance to other biotic stresses have been identified, namely to groundnut rosette virus, Sclerotinia blight, aflatoxin contamination, aphids, and tomato spotted wilt virus. Marker-assisted breeding is still in early stages, and development of more rapid and inexpensive markers from transcriptome and genome sequencing is expected to accelerate progress.

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Significance of Peanut

Peanut (groundnut) ranks second to soybean in the world market trade of legume oilseeds both in area grown and tonnage produced. Peanut is grown in more than 100 countries (Nwokolo 1996), with a total production of 37.7 million tons from 24.1 million hectares in 2010 (FAO 2012), with a mean productivity of 1.56 t/ha. The five largest producers in the world in 2010, based on pod tonnage, were China, India, Nigeria, the United States, and Senegal. Crop yield per hectare varies from region to region with the United States having the highest (3.7 t ha^{-1}) among major producers, followed by China (3.4 t ha^{-1}), Brazil and Argentina with values of 2.7 tons per hectare, and Senegal, Nigeria, and India with yields of 1.0 to 1.1 tons per hectare (FAO 2012). The crop is a rich source of oil (36–54%), proteins (16–36%), and carbohydrates (10–20%) (Knauft and Ozias-Akins 1995). Peanut is used for its seed, which supplies essential minerals such as zinc, iron, phosphorus, and calcium and vitamins such as riboflavin, thiamine, niacin, and vitamin E; peanut is also a major source of oil with benefits for human health (The Peanut Institute 2004). In some countries, the haulm is used as a source of fodder. Peanut, as a member of the Fabaceae (Leguminosae), is capable of converting atmospheric nitrogen into ammonia by symbiotic nitrogen fixation. Thus, in addition to being a food crop, peanut is capable of increasing the fertility of the soil (Pimratch et al. 2004) as a rotation crop. Peanut is important industrially; the famous scientist George Washington Carver identified more than 300 uses for peanut and peanut products over a century ago.

Peanut is well suited to contribute significantly to poverty reduction in the developing world, with a potential to accelerate the achievement of the United Nation's Millennium Development Goal of halving world poverty by 2015, because more than 90% of world production is realized in developing countries (FAO 2012). In Africa, women typically have the responsibility for post-harvest processing and sale of peanut, and thus improvement in yields is expected to improve the economic well-being of women (Kaaya and Christie 2007).

Genetic Structure of Peanut (Groundnut)

Origin of the Genus *Arachis* and Sections within the Genus

In 1753, Linnaeus described the domesticated peanut as *Arachis hypogaea*, depicting peanut as a weed with underground fruits, unlike most angiosperms. The *Arachis* genus was placed within the Leguminosae (Fabaceae) family. Within this family, the major grain legumes are in the Papillionoideae, which is further subdivided into several clades, among them are the Phaseoloids (milletoids or warm season legumes) that include the genera *Glycine*, *Phaseolus*, and *Vigna*, Galegoids (cool season legumes) including *Pisum*, *Medicago*, *Lens*, and *Vicia*, and the Genistoids, which include *Lupinus* (Doyle and Luckow 2003; Lewis et al. 2005). *Arachis* is distinct from these, belonging to the Dalbergoids, which includes peanut and *Stylosanthes*.

According to Gregory and Gregory (1979) and Krapovickas and Gregory (1994), the genus developed in the southwestern part of Mato Grosso do Sul, Brazil or northeast Paraguay, because what appeared to be the morphologically most ancient species of the genus, *A. guaranitica* Chodat. and Hassl. and *A. tuberosa* Bong. ex Benth., are still growing in that area. This would be consistent with *Stylosanthes* being the progenitor genus, in agreement with molecular phylogenetic work (Lavin et al. 2001). Wild species of *Arachis* have been collected in Brazil, Bolivia, Paraguay, Argentina, and Uruguay (Krapovickas and Gregory 1994; Singh and Simpson 1994; Jarvis et al. 2003).

Based on morphological and cross-compatibility data and geographic distribution, it has been proposed that the genus has evolved into species that fit into nine taxonomic sections (Krapovickas and Gregory 1994), which include the morphologically most ancient section *Trierectoides* with its two species with three

leaflets, *A. tuberosa* and *A. guaranitica*. From these ancient progenitors developed the sections *Erectoides*, *Extranervosae*, *Triseminatae*, and *Heteranthae*. The species of these four sections have varying affinities to the primitive section, as reported by Gregory and Gregory (1979) and Krapovickas and Gregory (1994). The more advanced sections include the *Caulorrhizae*, *Procumbentes*, and *Rhizomatosae*. The affinities of these latter species groups are varied as well, but with very limited successes reported in crossing with species of the most advanced section, *Arachis* (Gregory and Gregory 1979; Krapovickas and Gregory 1994). Krapovickas and Gregory (1994) described 69 species and Valls and Simpson (2005) added descriptions of 11 more, for a total of 80 species. There are at least 11 more species that have been collected but not yet described (Valls 2011).

Section *Arachis*

The *Arachis* section is the most advanced of the 9 sections and encompasses 31 described species, including the cultigen, *A. hypogaea* and one other cross-compatible tetraploid species, *A. monticola* Krapov. & Rigoni, plus 29 diploid annual and perennial species (Krapovickas and Gregory 1994; Valls and Simpson 1994; Valls and Simpson 2005). All but one of these species can be crossed to *A. hypogaea* and *A. monticola* with varying degrees of difficulty (Krapovickas and Gregory 1994; Singh and Simpson 1994). The distribution of the *Arachis* section has overlapped that of the other sections in many areas. It is not unexpected that the most advanced species would be more adaptable and thus colonize a larger geographical area. Also, people have played a role in the distribution of several species, most of which belong to section *Arachis*, including *A. stenosperma* and *A. hypogaea*. This latter species is the most widely cultivated member of the genus.

The remaining species of section *Arachis* are diploid and had been grouped until recently into three genomes (A, B, and D) each having 20 chromosomes. To date, 20 A-genome diploid species have been described (Krapovickas and Gregory 1994); among these are perennials *A. cardenasii*, *A. diogoi*, *A. helodes*, *A. villosa*, and *A. correntina* and annuals *A. duranensis* and *A. stenosperma*. Based on cytological evidence and cross-hybridization data, *A. cardenasii* was considered originally to be the most probable A-genome ancestor of *A. hypogaea* (Smartt et al. 1978). More recently, it has been proposed that this genome type can be divided into three groups based on karyotype (Robledo and Seijo 2010).

Initially only one annual B-genome species, *A. batizocoi*, was identified (Smartt et al. 1978), the B genome being associated with the absence of a specific small pair of A chromosomes (Fernandez and Krapovickas 1994). Accordingly, *A. batizocoi* was first proposed as the B genome donor to the cultigen (Smartt et al. 1978). However, cytological measurements discounted this hypothesis (Stalker and Dalmacio 1986). Subsequently, cross-compatibility, molecular, and cytological studies provided evidence for up to 10 B-genome species (Krapovickas and Gregory 1994; Kochert et al. 1996; Milla et al. 2005b; Tallury et al. 2005; Valls and Simpson 2005; Burow et al. 2009). However, the low pollen fertility, sterility, and separate molecular phylogenetic groupings of *A. ipaënsis* and *A. batizocoi* led Burow and coworkers to question whether *A. ipaënsis* and *A. batizocoi* belong to the same genome (Burow et al. 2009). Based on FISH, GISH, and geographic origin, Robledo and Seijo (2010) proposed that the B genome classification is not accurate and should be split into three genome types. *Arachis ipaënsis*, *A. magna*, *A. gregoryi*, *A. vallsii*, and *A. williamsii* are B genome *sensu stricto*, *A. batizocoi*, *A. cruziana* and *A. krapovickasii* being reclassified as K genome, and *A. benensis* and *A. trinitensis* as F genome.

The D genome consists of one species, *A. glandulifera*. This species is characterized by extensive genome rearrangements relative to other section *Arachis* species, as observed cytologically (Stalker 1991).

In addition, there are three diploid species that possess 18 instead of 20 chromosomes. These

species have been described as *A. decora*, *A. palustris*, and *A. praecox* (Lavia 1996, 1998; Peñaloza and Valls 2005; Valls and Simpson 2005).

Origin of Arachis Hypogaea

A. hypogaea is a tetraploid (2n=4x=40) (Husted 1936), and the only other known tetraploid species in the section, *A. monticola*, is closely related to it. Hybridization between the cultigen and section *Arachis* diploids is possible, but no evidence has been found that this has contributed to ongoing gene flow into the cultigen in nature. Cultivated peanut is considered to be an AB tetraploid, arising from hybridization between A and B diploid species (Smartt et al. 1978).

Lack of marker polymorphism in the cultigen using RFLP and RAPD markers (Halward et al. 1991; Kochert et al. 1991) contributed to the hypothesis that all varieties and botanical types of *A. hypogaea* share common diploid progenitors (Kochert et al. 1996). RFLP analysis determined that *A. duranensis* had greater similarity to *A. hypogaea* than did *A. cardenasii* (Kochert et al. 1991, 1996), and *A. duranensis* is considered by many now to be the likeliest A-genome ancestor. However, subsequent marker analyses have also proposed *A. villosa* (Raina and Mukai 1999), *A. helodes*, and *A. simpsonii* (Milla et al. 2005b) as potential A-genome donors.

Evidence from archaeological data (Simpson and Faries 2001), molecular marker data (Kochert et al. 1991, 1996), fluorescent *in situ* hybridization analysis using rDNA as labeled probe (Raina and Mukai 1999; Seijo et al. 2004), and gene sequence data (Jung et al. 2003; Ramos et al. 2006) strongly supported *A. ipaënsis* instead of *A. batizocoi* as B genome donor. However, new discoveries of wild *Arachis* species are still being made (Valls and Simpson 2005), and it is possible that other candidates could be discovered.

As a result of explorations by the Spanish and Portuguese, peanut spread quickly from the Americas to other parts of the world since the 16th century. Wynne and Coffelt (1982) indicated the existence of an important secondary center of diversity within *A. hypogaea* in Africa, where a large amount of variation is thought to arise from hybridization and selection in different environments.

Krapovickas and Gregory (1994) classified *A. hypogaea* into two subspecies and six botanical varieties. *A. hypogaea* subsp. *hypogaea* is characterized by a spreading growth habit, alternating vegetative and reproductive nodes, lack of flowers on the mainstem, medium-to-large seeds, medium-to-late maturity, and includes the botanical varieties *hypogaea* (virginia and runner market types) and the less frequently cultivated *hirsuta*. Several genotypes cultivated among native Brazilian Indians from the Xingu region have been characterized morphologically and using molecular markers and are reported to group with the *hypogaea* subspecies (Freitas et al. 2007). The *fastigiata* subspecies is typified by erect growth habit, sequential reproductive nodes, flowers on the mainstem, small seeds, and early maturity. These include the botanical varieties *fastigiata* (valencia), *vulgaris* (spanish), *peruviana*, and *aequatoriana*. The latter two are not cultivated widely outside of Peru, northwestern Brazil, northern Bolivia, and Ecuador.

Introgression Pathways

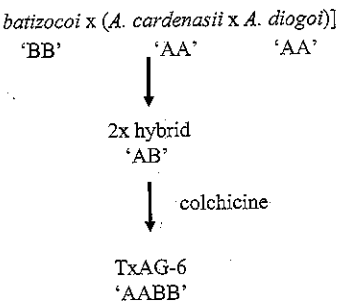
Attempts to utilize wild species as sources of new alleles have been met with limited success because of genomic (A and B genomes) and ploidy (diploid and tetraploid) barriers (Stalker and Moss 1987). Several pathways have been attempted with varying degrees of success, of which this chapter covers three: the hexaploid route, the autotetraploid route, and the allotetraploid route (the latter more commonly known simply as the tetraploid route).

The hexaploid route involves crossing a diploid wild species with *A. hypogaea* to generate a sterile triploid hybrid, followed by doubling

the hybrid chromosome number with colchicine to the hexaploid level (60 chromosomes). The progeny is backcrossed repeatedly to *A. hypogaea* until the progeny regained the normal chromosome number of 40. This method was used in crosses between *A. hypogaea* and seven diploid species, among them *A. cardenasii* (Smartt and Gregory 1967; Smartt et al. 1978; Stalker and Moss 1987; Wynne and Halward 1989). The progeny of the *A. hypogaea* x *A. cardenasii* cross have been used for development of marker maps, introgression populations, and germplasm releases (Stalker et al. 2002a, 2002b) or varieties with nematode, rust, and late leaf spot resistance, such as GPBD-4 (Gowda et al. 2002) (see discussion that follows).

The autotetraploid route involves the treating of two wild diploid species with AA and BB genomes types with colchicine to create synthetic autotetraploids. The synthetic auto tetraploids, with genomic composition AAAA or BBBB, are crossed to obtain plants with genotype AABB. Three autotetraploids were generated by Singh (1985) and crossed to *A. hypogaea*. Fertility of the autotetraploids varied, but fertilities of progenies backcrossed by *A. hypogaea* were higher.

The allotetraploid route involves the creation of synthetic amphidiploids by crossing two diploids of different genomes, followed by doubling with colchicine to the tetraploid level. This method was used to develop the TxAG-6 breeding line (Simpson 1991; Simpson et al. 1993), although by a slight variation of the procedure (Figure 1).



The hybrid TxAG-6 was backcrossed repeatedly to recover the cultivated phenotype to develop various varieties most notably incorporating resistance against root-knot nematode (see discussion later in the chapter).

Since the development of TxAG-6, a number of new synthetic amphidiploids have been created. For example, in a probable "resynthesis" of *A. hypogaea*, an amphidiploid was made from *A. ipaënsis* and *A. duranensis* (Fávero et al. 2006). From this amphidiploid, a series of structured introgression lines and agronomically adapted selected lines with some level of late leaf spot resistant have been made (Foncéka et al. 2009; Galhardo et al. 2011). Subsequently, additional amphiploids have been developed (Fávero et al. 2011; Leal-Bertioli et al. 2011; Santos et al. 2011). Almost all had greater resistance to leaf spot and rust than the cultivated species, with the most resistant amphiploids being *A. magna* x *A. cardenasii*, *A. magna* x *A. stenosperma*, *A. batizocoi* x *A. stenosperma*, and *A. gregoryi* x *A. stenosperma* (Fávero et al. 2011; Leal-Bertioli et al. 2011).

Genetic Linkage Maps of *Arachis*

Molecular Markers for *Arachis*

The development of molecular markers for peanut has followed the technical trends of the times. The first studies were based on isozymes and proteins (Krishna and Mitra 1988; Grieshammer and Wynne 1990; Lu and Pickersgill 1993), followed by Restriction Fragment Length Polymorphism—RFLPs (Kochert et al. 1991; Paik-Ro et al. 1992; Kochert et al. 1996), Random Amplified Polymorphic DNA—RAPDs (Halward et al. 1991, 1992; Hilu and Stalker 1995; Subramanian et al. 2000), Amplified Fragment Length Polymorphism—AFLPs (He and Prakash, 1997; Gimenes et al. 2000; He and Prakash 2001; Gimenes et al. 2002; Herselman, 2003; Milla et al. 2005a, 2005b; Tallury et al. 2005), and more recently microsatellite markers (Hopkins et al. 1999; Palmieri et al.

2002; He et al. 2003; Ferguson et al. 2004; Moretzsohn et al. 2004; He et al. 2005; Moretzsohn et al. 2005; Palmieri et al. 2005; Bravo et al. 2006; Budiman et al. 2006; Gimenes et al. 2007; Proite et al. 2007; Wang et al. 2007; Cuc et al. 2008; Naito et al. 2008; Liang et al. 2009; Moretzsohn et al. 2009; Song et al. 2010; Yuan et al. 2010; Koilkonda et al. 2012; Macedo et al. 2012; Pandey et al. 2012) and molecular markers based on MITE markers (Shirasawa et al. 2012 and unpublished data). Generally, these markers have shown a trend toward becoming more informative, and now microsatellites, being codominant and easy to score in the tetraploid genome, are considered the molecular marker of choice, with MITE markers also showing much potential.

Maps Based on Crosses Involving Wild Species

The very narrow genetic base of cultivated peanut has provided a substantial obstacle to genetic mapping using only cultivated germplasm. This meant that maps were initially generated using crosses involving wild species. Subsequently mapping in cultivated x cultivated crosses has advanced considerably (see discussion later in the chapter). In spite of this, mapping using crosses involving wild species is likely to continue to be important. Wilds are a source of new alleles for cultivated peanut conferring, for instance, strong disease resistances; the greater DNA polymorphism of the wilds allows for higher resolution mapping; also, diploid genetics simplifies genetic analysis and the use of some marker types (notably marker types based on single nucleotide polymorphisms, or SNPs).

The first genetic linkage map of peanut was developed using an F_2 population of a cross between A-genome diploids *A. stenosperma* and *A. cardenasii*. The 117 mapped RFLP markers were distributed among 11 linkage groups over 1,063 cM (Halward et al. 1993). A second map was constructed from a tetraploid cross of the cultivar Florunner × the synthetic amphidiploid TxAG-6 {*A. batizocoi* × [*A. cardenasii* × *A. diogoi*]}^{4x}. A total of 370 RFLP loci were mapped onto 23 linkage groups, for a map distance of 2,210 cM (Burow et al. 2001). The map was characterized by pairing of homoeologous linkage groups, consistent with a disomic nature of the cultigen. An AFLP-based A-genome map was generated from an F_2 population developed from the cross *A. kuhlmannii* x *A. diogoi*; 102 markers were mapped over 1,068 cM (Milla 2003). A RAPD-based map of *A. stenosperma* x *A. cardenasii* was developed by Garcia et al. (2005). This map contained 167 RAPD and 39 RFLP loci spanning 800 cM and 11 linkage groups.

The first microsatellite-based map of peanut was developed with an F_2 population derived from a cross between A genome diploids *A. duranensis* and *A. stenosperma*, and had 170 microsatellite markers on 11 linkage groups covering 1,231 cM (Moretzsohn et al. 2005). Subsequently a microsatellite map of the B genome based on a cross of *A. ipaënsis* and the closely related *A. magna* was produced (Moretzsohn et al. 2009). This map had 10 linkage groups, with 149 loci spanning a very similar total map distance of 1294 cM. The comparison of 51 shared markers between these two maps revealed high levels of synteny, with all but one of the B linkage groups showing a single main correspondence to an A linkage group. Foncéka et al. (2009) developed a map of 289 SSR markers using a BC_1 population between the cultivar Fleur 11 and a synthetic amphidiploid (*A. duranensis* x *A. ipaënsis*)^{4x}. This map again showed good colinearity between the A and B subgenomes in general, though several inversions of order were noted.

A higher-density version of the diploid map based on the cross of *A. duranensis* and *A. stenosperma* published by Moretzsohn et al. (2005) was reported by Leal-Bertioli et al. (2009). This map consisted of a total of 369 markers, including 188 SSRs and 80 legume anchor markers, 46 AFLPs, 32 NBS analogs, 17 SNPs, 4 RGA-RFLPs, and 2 RGA-SCAR

markers. Virtually all markers on this map were sequence characterized. This, in combination with the high proportion of low or single-copy gene markers allowed the map to be aligned to the fully sequenced genomes of *Lotus japonicus* and *Medicago truncatula* (Sato et al. 2008; www.medicago.org). These alignments revealed surprising degrees of synteny considering the time of species divergence (estimated at about 55 million years). Phylogenetically *Arachis* is an outgroup to *Medicago* and *Lotus*, and for this reason, comparisons are particularly informative for making evolutionary inferences. Using genome plots *Arachis* versus *Lotus*, *Arachis* versus *Medicago*, and comparing to a previously published plot between *Lotus* and *Medicago* genomes (Cannon et al. 2006; Bertioli et al. 2009), 10 distinct conserved synteny blocks and also non-conserved regions could be observed in all genome comparisons (Bertioli et al. 2009). This clearly implies that certain legume genomic regions are consistently more stable during evolution than others. It is notable that these regions are large scale, and apparently in some cases consist of entire chromosomal arms.

Intriguingly, an analysis of the retrotransposon distributions in *Lotus* and *Medicago* shed further light on these observations. Retrotransposons are unevenly distributed in both *Lotus* and *Medicago*, and retrotransposon-rich regions tend to correspond to variable regions, intercalating with the synteny blocks, which are relatively retrotransposon poor. Furthermore, while the variable regions generally have lower densities of single-copy genes than the more conserved regions, some harbor high densities of the fast-evolving disease resistance genes (Bertioli et al. 2009). For *Arachis* it was notable that LGs 2 and 4, which harbor the most prominent clusters of resistance gene analogs (RGAs) and QTLs for late leaf spot resistance, showed shattered synteny with both *Lotus* and *Medicago*. An association between RGAs and retrotransposons in *Arachis* has also been supported by studies on two peanut retrotransposons FIDEL and Matita (Nielen et al. 2010, 2011).

Genetic Maps Based on Cultivated x Cultivated Crosses

Screening of isozyme, RFLP, and RAPD markers on accessions of *A. hypogaea* identified only very low levels of polymorphism among cultivated peanut accessions (Kochert et al. 1991; Halward et al. 1992; Lu and Pickersgill 1993; Burow et al. 1996; Subramanian et al. 2000; Dwivedi et al. 2001). The partial first linkage map from a cross between accessions of *A. hypogaea* was constructed using an F_2 population (Herselman et al. 2004). Five linkage groups with 12 markers spanning 139 cM of the genome were reported. The first reasonably complete genetic maps of cultivated peanut were published by Hong et al. (2008) and Varshney et al. (2009). Hong et al. (2008) tested 1,048 SSR primer pairs and mapped 131 SSR loci onto 20 linkage groups for a total length of 670 cm on an RIL population between the cultivars Yueyou 13 and Zhenzhuhei. Varshney et al. (2009) screened 1,145 SSR markers and mapped 135 loci onto 22 linkage groups spanning 1,271 cM onto an RIL population developed from two parental genotypes, TAG 24 and ICGV 86031. Later a composite map containing 175 SSR markers in 22 linkage groups was developed from three cultivated crosses (Hong et al. 2010); of 901 primer pairs screened, 146, 124, and 64 were polymorphic. The most saturated map so far was recently published by Wang et al. (2012), containing 385 polymorphic SSRs covering 318 loci.

Attempts to develop maps with higher densities have required screening several thousand SSR markers. The SSR-based cultivated genetic map with 135 marker loci developed by Varshney et al. (2009) was then further saturated up to 191 SSR loci (Ravi et al. 2011). Two new partial genetic maps with 56 (TAG 24 × GPBD 4) and 45 (TG 26 × GPBD 4) marker loci (Khedikar et al. 2010; Sarvamangala et al. 2011) were constructed covering genome distances of merely 462.24 and 657.9 cM, respectively. These two maps were then saturated with enhanced genome coverage up to 188 (1,922.4 cM) and

181 (1,963 cM) marker loci, respectively, along with construction of a consensus map based on these two populations segregating for foliar disease resistance with 225 SSR loci and a total map distance of 1,152.9 cM (Sujay et al. 2011). In addition to the aforementioned three saturated maps, more recently two more genetic maps based on RIL populations segregating for traits related to drought tolerance, namely ICGS 76 × CSMG 84-1 (119 SSR loci) and ICGS 44 × ICGS 76 (82 SSR loci), were developed with genome coverage of 2,208.2 cM and 831.4 cM, respectively. Since the aforementioned three populations (TAG 24 and ICGV 86031, ICGS 76 × CSMG 84-1, and ICGS 44 × ICGS 76) were segregating for traits related to drought tolerance, a consensus map (2,840.8 cM) with 293 SSR loci was developed. All the parentals were cultivated genotypes, except for GPBD-4, which is predominantly cultivated with some *A. cardenasii* parentage derived through the hexaploid route (Smartt et al. 1978; Gowda et al. 2002). They observed 6-10% polymorphism for different marker types and mapped 652 markers into a high-density composite map based on all the five populations. More recently, Qin et al. (2012), after screening a total of 4,576 markers, identified 260 and 181 polymorphic markers, respectively, for the two RIL populations, namely Tifrunner × GT-C20 (T population) and SunOleic 97R × NC94022 (S population). Individual genetic maps were constructed for T and S populations with 236 and 172 marker loci, respectively. An integrated map was then constructed with 324 marker loci covering 1,352 cM genome distance (Qin et al. 2012). For the creation of the highest-density map of cultivated peanut to date, with more than 1,000 markers, screening was done by *in silico* analysis of DNA sequence data from the parentals (Shirasawa et al. 2012).

SNP-Based Maps of Peanut

Two significant SNP-based maps exist for peanut. The first is an extension of the A-genome diploid map of Moretzsohn et al. (2005) to 165 SSR, 78 anchor markers, 17 RGA, and 507 SNP markers (Gouvea 2012). A second SNP-based map of peanut has been reported by Nagy et al. (2010), wherein a high-density genetic map of the A genome was developed from an intraspecies cross within *A. duranensis*, and 971 SSRs, 221 single-stranded DNA conformation polymorphism (SSCP) markers, and 1,127 SNPs were mapped. Extension of SNP-based maps to the tetraploid has not been accomplished yet, and will require separation of A- and B-genome sequences, but is expected to greatly accelerate genetic mapping and marker-assisted selection when available.

Resistance Gene Analogs

Plant resistance genes have been found to fall into several classes, among which genes encoding the nucleotide binding site (NBS) are the most characterized. The NBS domain is thought to act in signal transduction pathways.

Using conserved amino acid motifs, degenerate primers can be designed that are able to amplify RGAs from any plant species (see Hammond-Kosack and Parker 2003). Using such primers, RGAs have been identified from wild and cultivated peanut. Seventy-eight nonredundant NBS-encoding regions were characterized by Bertioli et al. (2003). Phylogenetic analysis of these sequences with NBS encoding sequences from *Arabidopsis thaliana*, *Medicago truncatula*, *Glycine max*, *Lotus japonicus*, and *Phaseolus vulgaris* showed that most *Arachis* NBS sequences fall within legume-specific clades, and that sequences in some clades appear to have undergone extensive copy number expansions in the legumes. This underlines the apparent quickly evolving nature of resistance gene analogs. An additional 234 sequences were identified and mapped onto 250 nonredundant BAC clones containing NBS-encoding sequences (Yüksel et al. 2005). More recently, 401 RGAs were mined from a peanut

EST database, of which 2 were mapped (Liu et al. 2012).

As regards the genetic architecture of disease resistance genes, candidate genome regions that control disease resistance were identified by Leal-Bertioli et al. (2009). For this, 34 sequence-confirmed candidate disease resistance genes and five QTLs for resistance against late leaf spot were mapped in a diploid *A. duranensis* x *A. stenosperma* cross. Candidate genes and QTLs were distributed on all linkage groups except for the smallest, but the distribution was not even. Groupings were apparent on the upper region of linkage group 4 and the lower region of linkage group 2, indicating that these regions are likely to control disease resistances. As noted previously, these candidate regions showed shattered synteny with *Lotus* and *Medicago*, indicating that RGA-containing regions are probably faster evolving than some other genome regions. In a different study, resistance to root-knot nematode from the wild diploid *A. cardenasii* was mapped to the A genome linkage group 9 (Nagy et al. 2010). This region is particularly interesting genetically because it displays strongly suppressed recombination with the A genome of *A. hypogaea* and appears to cover about one-third to a half of a chromosome. Recently Ratnaparkhe et al. (2011) sequenced two peanut BACs containing six RGAs and concluded that synteny was not high with *Lotus*, *Medicago*, or *Arabidopsis*, and that there was evidence of intergenic and intragenic gene conversions and unequal crossing-over in this region in peanut.

Marker-Assisted Breeding of Peanut

Nematode Resistance: A Case Study in the Effectiveness of Markers in Breeding for a Simply Inherited Trait

Etiology

Meloidogyne species (root-knot nematode) are the most important nematode species limiting yield in peanut (Porter et al. 1984). Of these, the predominant pathogenic species to peanut are *M. arenaria* (Neal) Chitwood, *M. hapla* Chitwood, and *M. javanica* (Treub) Chitwood. *Meloidogyne haplanaria* (Eisenback et al. 2003) is a peanut parasite with limited distribution in the United States. Root-knot nematodes are found on the commercial peanut in many parts of the world, with *M. arenaria* being the predominant pathogenic species in the southern United States, especially in Alabama, Florida, Georgia, and Texas. *Meloidogyne javanica* is more common than *M. arenaria* on peanut in Africa and India (Tomaszewski et al. 1994). *M. hapla* has a cooler temperature optimum than *M. arenaria* or *M. javanica* and is referred to as the northern root-knot nematode. It is frequently found attacking peanut in the more northern areas of peanut production in the United States, specifically North Carolina, Oklahoma, and Virginia, and is also found on peanut in China. *Meloidogyne arenaria* and *M. javanica* are more aggressive pathogens than *M. hapla*, causing greater yield losses at lower nematode population densities (Koenning and Barker 1992; Abdel-Momen and Starr 1997).

The effects of *Meloidogyne* spp. are due to invasion of root tips cells by juvenile nematodes, followed by generation of giant cells in the roots as feeding sites, damaging the root system and impede nutrient transport in the plant (Caillaud et al. 2008). A plant gene conferring resistance to *M. incognita*, called *Mi*, was first isolated from tomato by positional cloning (Milligan et al. 1998), and encoded an NBS-LRR type protein. Other genes associated with response to nematode infection have been isolated by several researchers in different species (Lambert et al. 1999; Potenza et al. 2001). More recently, *M. arenaria*-challenged resistant species *A. stenosperma* (Guimarães et al. 2010; Morgante et al. 2011) identified many responsive genes. Two have been identified by RT-PCR to be upregulated upon infection.

High levels of resistance were identified in 11 of 15 diploid species tested against isolates of *M. arenaria*, and several accessions were also found with resistance to *M. hapla* (Nelson et al.

1989) and *M. javanica* (Stalker and Moss 1987). Since then, low to moderate levels of resistance were discovered in *A. hypogaea* (Holbrook and Noe 1992). Diploid crosses involving *A. cardenasii* demonstrated the presence of at least two dominant resistance genes (Starr and Simpson 1991). Resistance took the form of a hypersensitive response that inhibited the development of invading juveniles, and resulted in an almost total suppression of nematode reproduction. In *A. batizocoi* and *A. diogoi*, the mechanism of resistance could not be determined because of the difficulty in making interspecific test crosses with a susceptible parent, but resistance was associated with a lengthening in the time for juveniles to develop into adults and a decrease in the percentage of juveniles that reached adulthood (Nelson et al. 1990). Interestingly, the resistance of the resistant cultivar COAN, which is due to a single gene derived from *A. cardenasii*, is expressed as a failure of the invading nematode to initiate a functional feeding site in the vascular tissues, and many of the invading nematodes then emigrate from the roots or remain localized in the cortical tissues (Bendezu and Starr 2003). In the root-knot nematode-resistant *A. stenosperma*, penetration and development of the nematodes was dramatically reduced in comparison with that occurring in cultivated peanut. Neither giant cells nor nematodes developed beyond the second stage were found. Several cell features, including darkly staining cytoplasm and altered organelle structure, were observed in the central cylinder, indicating a hypersensitive-like response (HR) of infested host cells (Proite et al. 2008).

Breeding

Before introgression of resistance alleles from wild species, no root-knot nematode-resistant peanut cultivars were released, for lack of known sources of resistant germplasm. Root-knot nematode resistance was introduced into *A. hypogaea* from two crosses, that of *A. hypogaea* x *A. cardenasii* via the hexaploid route (Garcia et al. 1996) and by crosses among diploids followed by doubling with colchicine to the tetraploid level (Simpson 1991). The nematode-resistant cultivar COAN was the first peanut cultivar that contained a distinct trait donated from wild species (Simpson and Starr 2001). COAN was developed from the TxAG-6 amphidiploid, crossed to Florunner and advanced by five cycles of backcrossing followed by selfing and selection for root-knot nematode resistance (Simpson and Starr 2001).

Markers and Use in Selection

The first markers for an agronomically useful trait in peanut were for resistance to root-knot nematode (*M. arenaria*) from *A. cardenasii*. Two closely linked sequence characterized amplified region (SCAR) markers were identified for genes for reduced galling and egg number (Garcia et al. 1996). Simultaneously, three RAPD markers were associated with nematode resistance in several backcross breeding populations derived from the interspecific hybrid TxAG-6 [*A. batizocoi* × (*A. cardenasii* × *A. diogoi*)]^{4x} (Burow et al. 1996); however, these were all for the same gene, and although these did provide flanking markers, the one marker opposite the other two did not appear to be qualitatively inherited, but appeared to differ quantitatively in amplification, and was thus deemed too difficult to score accurately for marker-assisted selection (MAS). Instead, two (non-flanking) RFLP markers ca. 4cM from the resistance gene were developed by bulked segregant analysis (Church et al. 2000). The use of non-flanking markers was in part the result of a large gap (>30cM) (Burow et al. 2001) between markers on the other side of the gene.

MAS was used for the development of NemaTAM, the second nematode-resistant peanut cultivar (Simpson et al. 2003). The variety COAN had superior yield under disease pressure but had low yield under disease-free conditions. Two additional generations of backcrossing accompanied by the use of RFLP markers were used for the development of NemaTAM.

NemaTAM had the same markers for nematode resistance as were present in COAN, but it and other selected breeding lines had mean yields under disease-free conditions that were 135% to 160% higher than COAN had (Church et al. 2000). It was concluded that the linkage between resistance and low yield had been broken. However, scores of flanking markers were unavailable, and as such, it was never demonstrated whether the difference between COAN and NemaTAM resided on the chromosome containing the resistance gene or on a different chromosome.

Several benefits were observed in use of markers for development of the nematode-resistant variety NemaTAM (Choi et al. 1999; Church et al. 2000; Simpson et al. 2003; Cason et al. 2010). It was demonstrated that use of markers was more efficient than phenotypic selection, because plants selected by markers for the homozygous resistance gene bred true, unlike materials selected based on phenotype (which included heterozygous plants). Also, markers were more accurate, because phenotypic selection was accompanied by a certain amount of escapes. Although MAS would be affected by recombination between marker and the trait being scored, this rate of crossing over was less than the rate of assigning incorrect phenotypes. In addition, scoring could be performed on collected leaf tissue, eliminating the need to harvest the plant to perform nematode egg counts. However, the use of the RFLP marker was costly and required a large amount of DNA, radioisotope, and a long time (one to four weeks) before developing the X-ray film. Church et al. (2000) were able to determine the genotype of only 65-86% of the individuals attempted because of technical difficulties, such as the low quality or quantity of DNA, incomplete digestion of DNA, or poor hybridization or background on Southern blots. A nonisotopic method was used by Muitia et al. (2006), but this was more cumbersome and expensive than the use of radioisotope.

MAS was also used in development of a nematode-resistant, high-oleic variety from Tifguard. Tifguard is a nematode-resistant cultivar that also has resistance to tomato spotted wilt virus (Holbrook et al. 2008). Because of the cost, and difficulties associated with the RFLP marker technology, Tifguard was developed using standard phenotypic selection methods, using COAN as donor parent for nematode resistance (Holbrook et al. 2008). It would be desirable to have a high oleic peanut cultivar with the disease-resistant package available in Tifguard. Based on the development of improved molecular markers, it was decided to use MAS to develop Tifguard High O/L.

Improved markers were developed for the root-knot nematode resistance gene, and were used in development of Tifguard High O/L. Nagy et al. (2010) performed high-resolution mapping of nematode resistance with breeding material derived from the synthetic tetraploid pathway in comparison with an A-genome diploid species map. Twelve polymorphic markers and a previously published sequence characterized amplified region marker S197 (Chu et al. 2007a), developed from the published sequence of RAPD marker RKN440 (Burow et al. 1996), were found to be tightly linked with Rma in populations from two tetraploid crosses. During the breeding procedure to develop Tifguard High O/L, a dominant marker S197 (resistant allele), a dominant CAPS marker 1169/1170 (susceptible allele), and a codominant simple sequence repeat (SSR) marker GM565 (Nagy et al. 2010) were used (Chu et al. 2011). This allowed for the identification of homozygous resistant, homozygous susceptible, and heterozygous individuals.

Two homoeologous genes (*ahFAD2A* and *ahFAD2B*) encode for the key enzyme regulating the O/L ratio in peanut (Ray et al. 1993). Double recessive mutants are needed for the expression of the high O/L trait. A mutation in the *ahFAD2A* is prevalent in *A. hypogaea* subsp. *hypogaea* (Chu et al. 2007b), and all parents used in the development of Tifguard High O/L carry this mutant allele. A cleaved amplified polymorphic sequence (CAPS) marker 1101/1048 (Chu et al. 2009) was used to identify breeding lines

containing the mutant allele for the *ahFAD2B* gene. During the process of developing Tifguard High O/L, this CAPS marker was converted to a gel-free single nucleotide polymorphism (SNP) assay using HybProbe design (Chu et al. 2011).

An accelerated backcross breeding program with MAS was used to develop Tifguard High O/L. Tifguard was used as the recurrent female parent and two high O/L cultivars were used as donor parents for the high O/L trait. F_1, BC_1F_1, and BC_2F_1 individuals carrying the marker alleles for both nematode resistance and high O/L were selected for use as male parents in the next round of crossing. BC_3F_1 seedlings heterozygous for high O/L were selected and allowed to self. Homozygous BC_3F_2 seedlings were identified as Tifguard High O/L. Three cycles of backcrossing were deemed adequate based on the high coefficient of coancestry between recurrent and the donor parents (Chu et al. 2011).

The effectiveness of selection of nematode resistance has been the most successful use of MAS in peanut to date. However, the use of a single gene trait that confers near-immunity may be subject to breakdown of resistance under high selection pressure, and has been cause for concern even before the release of COAN. Therefore, new sources of resistance for nematodes, such as amphidiploids derived from *A. stenosperma*, which is highly resistant to fungi and nematodes (Proite et al. 2008, Leal-Bertioli et al. 2010; Santos et al. 2011), would be a useful resource for peanut breeding.

The previously mentioned markers for nematode resistance (Burow et al. 1996) were identified using bulked segregant analysis. This is efficient for identifying markers with major effects but is less successful at identifying markers with smaller effects. Evidence for presence of a second, recessive resistance gene was provided by Church et al. (2005). QTL analysis of a segregating BC_3F_1 population developed from the TxAG-6 x Florunner cross has revealed the presence of three additional QTLs, with QTLs now from both A and B genomes (Burow et al. 2012). The previously known marker contributes more to the explanation of phenotypic variance than the newer markers; however, newer markers may be of use to develop a variety with a more durable resistance. It is possible that the presence of these additional genes for resistance could explain in part the linkage drag for yield observed in COAN.

Leaf Spot Resistance: Two Complex Traits Controlled by Many Genes

Etiology

The foliar diseases of early leaf spot (caused by *Cercospora arachidicola* S. Hori) and late leaf spot (caused by *Cercosporidium personatum* [Berk. and Curtis] Deighton), also known as *Phaeoisariopsis personata* ([Berk. and Curt.] Deighton), are two of the most limiting biotic stresses in peanut production known worldwide (Shokes and Culbreath 1997), causing yield losses of up to 50% (Smith 1984; McDonald et al. 1985). In West Africa, yield losses can be as high as 70% (Waliyar et al. 2000). Both diseases often occur together in the same field, even though one may predominate (Hassan and Beute 1977). The result of the disease is defoliation, reducing yield through reduction of photosynthesis, death of the plant, and pod loss.

Although these diseases can be controlled using fungicides, their application is costly in the United States (Coffelt and Porter 1986). A study in Ghana (Naab et al. 2005) has confirmed that foliar application of fungicides can increase biomass and kernel yields in rainfed peanuts by 39% and 75%, respectively. However, the use of fungicides, though allowing to increase yields, is not feasible for many farmers in West Africa, where poverty is prevalent. Credit facilities for the purchase of inputs, as well as the input availability and delivery system, are not adequately developed. The most practical control method for these farmers would be the use of host plant resistance (Holbrook and Stalker 2003).

Breeding

Some wild peanut species have considerable resistance to leafspots. Subrahmanyam et al. (1989) evaluated 96 accessions of wild *Arachis* species for reactions to late leaf spot and observed that lesions were formed on all accessions, but lesions were small and nonsporulating on all accessions of sections *Erectoides*, *Triseminatae*, *Extranervosae*, *Rhizomatosae*, and *Caulorrhizae*. Lesions with diameters ranging from 0.16 to 1.0 mm were, however, found in section *Arachis*. In section *Arachis* it was further observed that 15 accessions had no sporulating lesions, and sporulation was slight to extensive in other accessions. In *A. stenosperma*, the failure of late leaf spot and rust pathogens to infect has been shown to be due to inability of the fungal hyphae to penetrate the stomata of peanut leaves (Leal-Bertioli et al. 2010). Rao et al. (2003) list seven wild species (held at The International Crops Research Institute for the Semi-Arid Tropics, ICRISAT) that have resistance to early leaf spot (ELS), nine to late leaf spot (LLS), and twelve to rust that have been exploited to develop breeding lines with some degree of resistance (Simpson et al. 1993; Stalker et al. 2002a). In Brazil, IAC-Caiapó, a runner-type cultivar, shows moderate resistance to foliar diseases and high productivity (Godoy et al. 1999).

All commercially grown cultivars used to be susceptible to some extent to both diseases (Shokes and Culbreath 1997), suffering yield losses of around 50% in the absence of fungicide sprays (Smith 1984; McDonald et al. 1985; Waliyar et al. 2000). It was not until 1984 that the first commercial U.S. cultivar (Southern Runner) with an appreciable level of resistance to late leaf spot was released (Holbrook and Stalker 2003). Cultivars with moderate levels of resistance such as Florida MDR 98 and C-99R (Gorbert and Shokes 2002a, 2002b) were later released. These have medium-to-late maturity. ICRISAT has also released several *A. hypogaea* accessions with some resistance to leafspots (Upadhyaya et al. 2001; Singh et al. 2003; Mathews et al. 2007), several with high yield as well as resistance to leafspots. Substantial progress has also been made in Ghana with collaboration between the Savanna Agricultural Research Institute (SARI) and ICRISAT, which has resulted in the release of three varieties, Edorpo-Munikpa, Nkatiesari, and Kpaniele (Frimpong et al. 2006; Padi et al. 2006). These are bunch-type, medium-to-late maturing (120 days) with resistance to both leaf spots.

Resistance has different components, including percent defoliation, incubation (time from inoculation until the appearance of lesions) period, latency (time from inoculation until sporulation) period, lesion number and diameter, sporulation, and pod yield (Green and Wynne, 1986; Chiteka et al. 1988a, 1988b; Anderson et al. 1993; Waliyar et al. 1993, 1995). Some have argued that as yield is the primary objective in any resistance program, selection based on defoliation would probably be the most appropriate due to its high heritability and the fact that reductions in yield are attributed in large part to premature defoliation in diseased fields (Anderson et al. 1991). Reports by Aquino et al. (1995) suggested that latency period and maximum percentage of lesions that sporulated were the components of resistance most highly correlated with late leaf spot disease development and suggested that using either component may facilitate more rapid selection of lines with improved levels of rate-reducing resistance in germplasm evaluations.

Wynne and Halward (1989) and Simpson (1991) suggested multigenic type resistance for the two leaf spot diseases with a strong possibility of each under the control of two or more genes. Available evidence indicates resistance to both early and late leaf spot to be quantitatively inherited (Sharief et al. 1978; Kornegay et al. 1980; Walls et al. 1985), with a large additive effect possibly with the involvement of cytoplasmic factors (Coffelt and Porter 1986). Resistance to late leaf spot is reported to be governed by five loci (Nevill 1982).

Some of the known components of resistance are subject to additive gene action (Sharief et al. 1978; Kornegay et al. 1980). Anderson et al. (1986) found significant differences among F_1 hybrids between relatively resistant and susceptible parents and argued that resistance to leaf spot may be controlled not only by recessive genes, but also by epistatic and additive alleles. In early leaf spot resistance studies, stability of resistance components has been found to vary across growing regions due to environmental interactions (Waliyar et al. 1993; Chiteka et al. 1997) as well as to differences in pathogen populations (Waliyar et al. 1993) or to both (Chiteka et al. 1997).

Heritability values for both diseases are reported to range from low to high depending on the resistance level of the parents used in the study, making selection in early generations ineffective in crosses resulting from parents with low heritabilities (Jogloy et al. 1987). Anderson et al. (1991) reported that values for broad-sense heritability for lesion number, sporulation, and defoliation rating for early leaf spot were 0.57, 0.16, and 0.56 while those for late leaf spot were 0.74, 0.54, and 0.88, respectively. For narrow-sense heritability for early leaf spot, the values were 0.18 and 0.53 for lesion number and sporulation. The corresponding values for late leaf spot were 0.74 and 0.26. Values for a second cross were generally lower in greenhouse studies, suggesting that dominance and epistatic genetic variance are substantial. In field studies, Iroume and Knauft (1987) obtained values of 0.16 to 0.26 for necrotic area and defoliation from segregating materials and attributed the variation between different crosses to relative differences in susceptibility levels of the parents used for each cross. Broad-sense (H_{bs}) and narrow-sense (h^2) heritability estimates for pod yield in peanut are reported to range from 28% to 82% and from 16% to 79%, respectively (Wynne and Gregory 1981; Wynne and Coffelt 1982). Combining ability estimates from a diallel cross indicated that GPBD-4 and ICG (FDRS) 79 were among the best parents for this trait. GPBD-4 was derived from a cross KRG 1 × ICGV 86855; the latter is an interspecific line (CS 16) developed previously from a cross between *A. hypogaea* and *A. cardenasii* (see Gowda et al. 2002; Stalker et al. 2002a).

High levels of resistance have also been associated with low yield, suggesting linkage or pleitropic effects (Iroume and Knauft, 1987), which means that breeding for high-yielding cultivars with resistance requires this linkage to be broken. Iroume and Knauft (1987) suggested this can be done in early generations under high disease pressure using an index that combines yield and disease severity traits. This was corroborated by Anderson et al. (1986) who reported effectiveness of selection in F_2 plants. Recently selection for leaf spot resistance, yield, and cultivated phenotype over four generations of progeny from a panel of BC_1s derived from *A. hypogaea* cv. IAC-Runner-886 x (*A. ipaënsis* x *A. duranensis*)^{4x} yielded 12 agronomically adapted lines with improved disease resistance compared to the recurrent parent (Galhardo et al. 2011). In this latter breeding scheme the apparently normal genetics of the progeny from this cultivated x wild cross was very helpful in obtaining the desired lines. In general, tapping useful alleles from wild species is hampered by linkage drag between desired and unadapted wild alleles requiring several cycles of backcrossing to recover most of the desirable agronomic traits. This is not only difficult but can be costly and time-consuming. Use of molecular markers can facilitate the identification, localization, and genetic dissection of loci that control quantitatively inherited traits such as yield (Tanksley and Nelson 1996) to speed up utilization of wild alleles.

Markers

Markers for additional traits have been developed. Stalker and Mozingo (2001) identified three RAPD markers associated with early leafspot lesion diameter in a peanut population derived from a cross between an *A. hypogaea*

x *A. cardenasii* introgression line and a cultivated variety. Two breeding lines developed from this material have been placed into advanced line trials.

Mapping of RFLP markers on BC_3F_1 lines in greenhouse studies identified five markers for leafspot resistance (Burow et al. 2008), including three QTLs for incubation period and one each for latency period, lesion number, and diameter. Those QTLs for latency period and lesion number were overlapping, suggesting linkage between the two or a QTL with pleiotropic effects. In addition, field evaluation of BC_3F_2 lines identified 29 markers for the domestication-related traits of main stem length, number of lateral branches, and pod and seed size (Burow et al. 2011).

Leal-Bertioli et al. (2009) reported the mapping of 34 RGAs and 5 QTLs for late leaf spot disease resistance on detached leaves of the F_2 plants of the A-genome mapping population derived from *A. duranensis* x *A. stenosperma*, and suggested additive or partial dominance gene action. One QTL explained almost half of the phenotypic variance observed. Two QTLs mapped near RGA markers. In a detailed QTL study based on cultivated genotypes, Khedikar et al. (2010) reported 11 QTLs for LLS; each QTL explained 2-7% of phenotypic variation in three environments, suggesting that the genes controlling LLS resistance in this cross are relatively minor. In maps from two populations, again using GPBD-4 as one parent, using a larger number (188 and 181) of markers and six trials, a major QTL for LLS was reported, which explained from 10% to 62% of phenotypic variance, depending on the environment; this appeared to give a bimodal (resistant/susceptible) distribution (Sujay et al. 2011). In all, 28 QTLs for LLS were identified.

These findings add to several others that leaf spot resistance in peanut is under the control of many genes and thus explains the difficulty in breeding for resistance. However, identification of a major QTL may allow for more rapid progress in transferring a significant degree of resistance from donor populations. Foncéka et al. (2009) concluded that the BC_1F_1 and BC_2F_1 interspecific hybrids resulting from their work should facilitate the development of advanced backcross and chromosome segment substitution breeding populations for the improvement of cultivated peanut, having used the putative progenitors of cultivated peanut from both the A and B genomes for the development of their interspecific amphidiploid. Combination of QTLs for agronomic and quality traits with those for leafspot analysis is expected to significantly accelerate breeding for resistance.

Rust Resistance: A Complex Trait that Could Be Simpler to Breed than Thought

Etiology

Rust (caused by *Puccinia arachidis* Speg.) is another important fungal disease that occurs widely in Africa and Asia and sporadically in North America and South America. It appears as a large number of small pustules on the underside of leaves, and in severe cases can cause significant defoliation and loss of yield. Overall, rust is generally less significant than leaf spots, even though occasionally outbreaks are severe and can cause severe losses. Rust frequently occurs in combination with leaf spots. Yield loss due to rust, in combination with early and late leaf spot diseases, can be particularly severe; in India, it is reported to be as high as 70% (Subrahmanyam et al. 1980, 1985).

Breeding

Resistance to rust, as also is the case for resistance to leaf spots, has been considered to be a quantitative trait. Resistance is measured as several components: leaf area damage percentage, infection frequency, incubation period, lesion diameter, and sporulation index. All measures were found to be positively correlated with one another, with the exception of incubation period, which was negatively correlated with the other measures (Mehan et al. 1994).

Resistance is present in *A. hypogaea*, with most of the resistant accessions being of subspecies *fastigiata* (Subrahmanyam et al. 1989). Inheritance studies indicated the presence of two or three recessive genes in some crosses, with evidence for epistatic interactions, and with resistance being accompanied by slowing down of disease development (Subrahmanyam et al. 1983b). Many wild peanut species were found to have strong resistance or immunity to rust, with evidence for dominance and additive epistatic interactions for resistance (Singh et al. 1984). In section *Arachis*, 11 diploid species accessions were immune and 3 were highly resistant; in sections *Erectoides*, *Extranervosae*, *Rhizomatosae*, and *Triseminate*, 37 of 38 accessions tested were immune (Subrahmanyam et al. 1983a). The tetraploid *A. monticola* was scored as susceptible. Further screening of 74 section *Arachis* accessions indicated that all had very low leaf damage, except for *A. monticola*, *A. ipaënsis*, and some accessions of *A. stenosperma* (Pande and Rao 2001). Breeding has resulted in release of some germplasm lines and varieties with improved rust resistance, sometimes also possessing resistance to late leaf spot (Gorbet and Shokes 2002a, 2002b; Singh et al. 2003).

Markers

Markers for rust in general have been discovered in the same populations analyzed for LLS mentioned earlier in this chapter. ICRISAT, in collaboration with University of Agricultural Sciences-Dharwad (UAS-D) in India, had identified and validated markers linked with these two foliar diseases. QTL analysis using a partial genetic map of a mapping population with 67 marker loci derived from the cross TAG 24 × GPBD-4 and multiple season phenotyping data on both the foliar diseases detected a total of 12 QTLs explaining between 1.7% and 55.2% of the phenotypic variation each (Khedikar et al. 2010). The SSR marker tightly linked to the major QTL (IPAHM103; QTLrust01) was then validated among a diverse set of genotypes as well as another mapping population (Sarvamangala et al. 2011) derived from the cross TG 26 × GPBD-4. Furthermore, the partial genetic linkage maps (TAG 24 × GPBD-4 with 67 marker loci and TG 26 × GPBD-4 with 53 marker loci) were both saturated to over 180 loci (Sujay et al. 2011). The populations were subjected to further phenotyping for seven to eight seasons. Final analysis detected a total of 15 QTLs for rust and 28 QTLs for LLS resistance (Sujay et al. 2011). These QTLs included a major QTL for LLS (QTLLLS01; linked markers GM1573 and pPGPseq8D09), which was detected across all the environments and explained between 10.27% and 62.34% of the phenotypic variation. In addition, three new SSR markers (GM1536, GM2301, and GM2079) significantly associated with the major rust QTL (QTLrust01) were identified (Sujay et al. 2011).

In parallel, the validated SSR marker (IPAHM 103) was deployed in initiating introgression of rust QTL into three elite groundnut varieties (ICGV 91114, JL 24, and TAG 24) using the donor GPBD-4 through marker-assisted backcrossing. Later, the newly identified linked markers (GM2079, GM2301, and GM1536) in the same QTL region have been used together with IPAHM103 for foreground selection to identify heterozygous plants at backcrossed F_1 generations (BC_1F_1, BC_2F_1 and BC_3F_1) and homozygous plants at backcrossed F_2 (BC_2F_2 and BC_3F_2) generations by S. Nigam and P. Janila of ICRISAT. As a result, 76 homozygous BC_3F_2 and 158 BC_2F_3 lines have been generated and screened for disease resistance during the rainy season of 2011 (Pandey et al. 2012). This initial screening has been encouraging and has lead to the identification of several promising lines showing remarkable reduction in disease symptoms.

Resistance to Other Diseases and Pests

Aphids

The aphid-transmitted groundnut rosette virus is an important pathogen of peanut in Africa and Asia. Groundnut rosette virus causes severe

stunting of the peanut plant and loss of yield. Until recently, there were no resistant cultivars, but resistant germplasm was identified (de Berchoux 1958, 1960; Subrahmanyam et al. 1998) and is being used for varietal development.

In an effort to identify markers for GRV resistance, Herselman et al. (2004) tested 308 AFLP primer combinations and were able to devise 5 linkage groups consisting of 12 markers; 1 marker was linked to aphid resistance.

Aflatoxin Resistance

Aflatoxin is a family of potent hepatotoxins and carcinogens that are also responsible for suppression of immune system function (Williams et al. 2004). Aflatoxin contamination occurs on several crops, including maize and peanut. The causative organism is *Aspergillus flavus*, which colonizes seeds and under certain conditions in the field and post-harvest storage may produce toxins.

Several attempts have been made to develop peanut varieties with low potential for developing aflatoxin, but this goal has been difficult to attain due to high variability in measurements, requiring up to 10 replications in the field. Eleven peanut accessions with at least a 70% reduction in aflatoxin have been identified (Holbrook et al. 2009), and advanced breeding lines have been developed. Additional materials have been identified (Nigam et al. 2009); however, high genotype x environment effects have been noted. Development of markers for this trait would be very useful in breeding.

Only one report exists to date on markers for resistance to aflatoxin contamination. Milla et al. (2005a) reported AFLP-based markers for *A. cardenasii*-derived resistance to aflatoxin contamination. Of 38 markers screened in the *A. hypogaea* x *A. cardenasii* population, 6 were found associated with aflatoxin concentration in the F_2 population at a low statistical threshold. Several proteins have been associated with infection of peanut with *Aspergillus* (Basha and Pancholy 1986). Luo et al. (2005) developed an EST-derived microarray of approximately 400 unigenes that were probed under different conditions. Twenty-five ESTs potentially associated with drought stress and response to *A. parasiticus* were identified. Subsequently, a microarray of 14,000 unigenes was developed from public peanut EST sequences (Kottapalli et al. 2009). Guo et al. (2011), using an oligoarray, profiled *Aspergillus flauvus* infection-responding genes in two contrast peanut genotypes. Additional work is still needed to find useful markers for aflatoxin resistance.

Tomato Spotted Wilt Virus (TSWV)

Tomato spotted wilt virus causes serious losses in the United States in fields where the virus is prevalent. The virus is transmitted by tobacco thrips (*Frankinellia* sp.) A segregating population of F_2 plants of an A-genome diploid cross *A. kuhlmannii* x *A. diogoi* was screened for resistance to TSWV, and five linked AFLP markers on one chromosome were associated with resistance at a high statistical threshold (Milla 2003; Milla et al. 2004). In Brazil, interspecific populations and wild species have also been found as promising for introgression of resistance to the thrips *Enneothrips flavens* (Janini et al. 2010).

Recently, one QTL each in Tifrunner x GT-C20 (T population) and SunOleic 97R x NC94022 (S population) crosses, explaining 12.9% and 35.8% phenotypic variance, respectively, was reported (Qin et al. 2012). The linked markers (IPAHM287 and Seq12F7) provide hope for marker-assisted improvement of this disease, but validation of markers as well as QTLs are required as these were identified based on single environment data.

Sclerotinia Minor

Sclerotonia blight (*Sclerotinia minor* Jagger) is a major problem in U.S. areas with cool autumns. The fungal form, sclerotia, can survive in the field for many years. Yield losses are typically about 10% but have been reported to be as high as 50% (Melouk and Backman 1995). Several resistant cultivars have been developed, but this

requires field screening; growth chamber screening can be done (Melouk et al. 1992) but does not work with all market types (Wilson 2008). By association analysis of 39 genotypes with 16 SSR markers, 1 SSR marker was found to be associated with resistance (Chenault et al. 2009). This marker was found to work in runner, spanish, and valencia market types but not in the virginia market type (Chamberlin et al. 2010). Using a transgenic approach, Livingstone et al. (2005) obtained peanut plants with increased resistance to *S. minor* by expressing a barley oxalate oxidase gene.

Conclusion

MAS in peanut has lagged behind other major crops. This is due in good part to the genetic bottleneck that occurred at tetraploidization, resulting in a limited amount of molecular variability detectable among accessions of the cultivated species. However, marker maps have been developed from wild species, and, to an increasing extent, the cultivated species using new marker types. It is expected that, with the increase in number of SSR markers and development of SNP-based markers, there will be greater use of MAS in both interspecific and cultivated accession crosses.

MAS has already proven itself to be useful in developing cultivars possessing resistance to the root-knot nematode, and is being used for selection for resistance to late leaf spot and rust, as well as for the high-oleic-acid trait. It is to be expected that, as the power of molecular tools increases and the cost decreases, MAS will be used to an increasing degree in this crop.

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