Molecular Markers of *Arachis* and Marker-Assisted Selection

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ABSTRACT

Many agronomic traits are difficult to select in *Arachis hypogaea* L. by conventional selection techniques, and marker-assisted selection offers an additional tool for obtaining improved germplasm lines. Molecular markers allow more efficient selection and offer a mechanism to eliminate undesirable traits associated with hybridizing diverse genotypes. The cultivated peanut has been analyzed by several marker systems, including RFLPs, RAPDs, AFLPs, and SSRs. Variation has been observed among diverse genotypes in approximately 5% of the markers analyzed, but the number is much lower between pairs of *A. hypogaea* lines. Conversely, a large amount of variation has been observed among *Arachis* species. Molecular maps have been constructed independently in two laboratories by utilizing *Arachis* species; however, a map of the cultivated peanut will be very difficult and costly to produce. Studies of advanced-generation interspecific hybrids have shown that *A. cardenasii* genes can be incorporatead into most linkage groups of *A. hypogaea,* indicating that *A. hypogaea* is not an allotetraploid in the classical sense where chromosomes from donor species are nonhomologous. Other molecular studies have identified *A. duranensis* and *A. ipaensis* as likely progenitor species of *A. hypogaea*. Associations of molecular markers with genes conditioning disease and insect resistances have been detected, and these investigations are beginning to be productive for selecting improved breeding lines and cultivars of peanut.

Key Words: AFLP, *A. hypogaea,* groundnut, isozymes, PCR, peanut, RFLP.

The cultivated peanut, *Arachis hypogaea* L., is a tetraploid ($2n = 4x = 40$) species native to South America. The species has been divided into two subspecies and six botanical varieties (Krapovickas and Gregory, 1994), and within each variety there are numerous variants for vegetative and reproductive traits (Stalker and Simpson, 1995). Cytological variation also has been observed within *A. hypogaea* (Stalker and Dalmacio, 1986), and two genomic groups have been described in the species. Self-pollination is most common in the genus, but up to 8% outcrossing can occur when large numbers of bee pollinators are present (Knauft *et al.*, 1992).

Wild species of *Arachis* are mostly diploid ($2n = 2x = 20$), but tetraploids ($2n = 4x = 40$) occur in two of the nine sections of the genus. Species are native to a large range of habitats, and both annuals and perennials occur in nature. Interest in the *Arachis* species has been directed toward better understanding of variation in the genus and identifying accessions with high levels of resistance to the many pests and pathogens that plague the cultivated peanut.

Marker-assisted selection has been a plant breeding tool since it was proposed by Sax in 1923 (Arus and Moreno-Gonzalez, 1993). The theory behind this method is that plant breeders could observe easy-to-score phenotypes to select difficult-to-score or low heritability traits that are linked to them (Tanksley, 1983). A good marker should (a) allow the separation of homozygotes from heterozygotes, thus allowing more genetic gain per generation than is possible without using the marker; (b) have early expression in the plant, thus saving time waiting for the desired phenotype to develop; and (c) not have interactions with other markers (Arus and Moreno-Gonzalez, 1993). Because successful plant breeding requires selecting many traits with complex inheritance, and desirable quantitative traits usually have both genetic and environmental components (Dudley, 1993), separation of these components to achieve maximum efficiency in breeding programs is necessary (Gebhardt and Salamini, 1992). Breeders originally depended on markers that had a morphological effect on the plant because these were the only markers available. However, most morphological marker types do not fit the description of a 'good' marker because they have either dominance effects, late expression, exist in epistatic relationships, or have deleterious effects on the plant (Tanksley, 1983).

With the development of molecular markers there has been great potential for increasing breeding efficiency because many of the marker systems have large numbers of polmorphisms; alternate alleles rarely have deleterious effects at the molecular or whole plant level; they are often codominant, allowing all genotypes to be distinguished in each generation; and they rarely segregate in epistatic ratios. Further, scoring of molecular markers does not depend upon gene expression and they are not affected by environment. Therefore, an accurate genotype can be established using any plant tissue at any developmental stage (Arus and Moreno-Gonzalez, 1993). The use of molecular markers can reduce the time and space necessary to evaluate plant populations; however, large numbers of markers must still be evaluated for associations with various traits, such as yield, disease resistance, flavor, etc., and linkages between markers and desired traits must be known for effective breeding (Dudley, 1993). A linkage map with many markers, especially when the genome is saturated with markers, can be used to locate genes of interest.

Isozymes, which are any two proteins that catalyze the same biochemical reaction but differ in chemical composition, were among the earliest molecular marker systems used for plant analyses. They can be extracted and separated by gel electrophoresis and polymorphisms in enzyme mobility may be used as markers (Weeden, 1989). Isozyme analyses of cultivated peanut have

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shown little variation (Grieshammer and Wynne, 1990), and these authors concluded that isozymes are not useful for characterizing polymorphism in *A. hypogaea.* However, isozyme polymorphisms are frequent among species of *Arachis* (Lu and Pickersgill, 1993; Stalker *et al.*, 1994) and between interspecific hybrids (Lacks and Stalker, 1993). Isozymes have been associated with agronomic traits in several crops but, similar to the studies in peanut, isozymes do not generate enough polymorphisms in most species to be useful for crop improvement (Weeden, 1989).

Restriction Fragment Length Polymorphisms (RFLPs) represented the first marker system that had a large number of polymorphisms. They are used widely both to create linkage maps and to implement indirect selection strategies. RFLPs can be used to study both recessive genes and multiple alleles.

RFLPs are produced by digesting DNA with restriction endonucleases that recognize a specific DNA sequence and then cleave the DNA strand in or near the sequence. Fragments thus produced can be separated by size on a gel electroporesis plate. Plants often produce so many fragments that the resulting gel is not interpretable. For complex genomes, a probe is made from cloned DNA that is homologous to a specific DNA sequence in the species being investigated. Radioactivity is used to label probes and bands are visualized when the unhybridized radioactivity is washed away and then an autoradiograph is produced. Sequences as rare as one in a million can be detected and any unique DNA sequence can be used as a probe as long as it binds with some part of the digested DNA fragments. Because RFLPs can be used to tag genes, they hold promise for making selections in a plant breeding program in large part because of reduce time needed to screen large populations in segregating generations.

In *A. hypogaea,* little molecular variation has been detected by using RFLP technologies (Kochert *et al.*, 1991) or exotic germplasm lines (Halward *et al.*, 1991). Kochert *et al.* (1996a) also reported that no variation was found between *A. hypogaea* and *A. monticola* Krapov. and Rigoni. However, significant amounts of variation have been observed among *Arachis* species (Kochert *et al.*, 1991; Paik-Ro *et al.*, 1992). RFLPs have been used to analyze species in section *Arachis* (representing taxa that will hybridize with *A. hypogaea*) and clusters that formed using multivariate analyses (Kochert *et al.*, 1991) correspond closely with morphological groups (Stalker, 1990); tetraploids were clearly separated from diploids in both investigations. Stalker *et al.* (1995) utilized RFLPs to examine genetic diversity among 18 accessions of *A. duranensis* Krapov. and W.C. Gregory and found a large amount of variation in the species. Individual accessions also could be uniquely identified by RFLP patterns. Kochert *et al.* (1996b) concluded that the cultivated peanut resulted from a cross between *A. duranensis* and *A. ipaensis* Krapov. and W.C. Gregory, and chloroplast analysis indicated that *A. duranensis* was the female progenitor of the cross.

An RFLP map was developed for peanut by analyzing an F_2 population from the diploid ($2n = 2x = 20$) interspecific cross of *A. stenosperma* Krapov. and W.C. Gregory (acc. HLK 410) and *A. cardenasii* Krapov. and W.C. Gregory (acc. GKP 10017). The linkage map covered 1063 cM with 117 markers in 11 linkage groups (Halward *et al.*, 1993). Fifteen unassociated markers also were reported. A second molecular map of peanut was created by Burow, Patterson, and Simpson using the tetraploid cross Florunner x 4x [*A. batizocoi* Krapov. and W.C. Gregory (*A. cardenasii* x *A. diogoi* Hoehne)] Burow (pers. commun.). Most of the 380 RFLP markers that have been mapped had disomic inheritance, with the exception of one linkage group which may be polysomic.

Polymerase Chain Reaction (PCR) technologies are based on annealing the extension of two oligonucleotide primers that flank a target region on the duplex of DNA (Erich *et al.*, 1991). The reaction is set up such that, as the DNA unwinds, each primer hybridizes to its complementary DNA sequence on each strand, and then DNA polymerase is used to extend the primer on each strand. The cycle of denaturation, primer hybridization, and DNA synthesis is used to exponentially multiply the number of copies of the target sequence. The advantage of PCR is that it requires only very small amounts of DNA and allows the DNA from a single extraction to be screened for hundreds of marker loci. One of the most widespread uses of PCR technologies is the random amplified polymorphic DNA (RAPD) methodology. No radioactivity is required and procedures are technically easy and rapid to perform as compared to RFLP methods (Waugh and Powell, 1992). The method is very sensitive to DNA polymorphisms, but there is a disadvantage that only dominant markers are detected.

In peanut, Halward *et al.* (1992) reported very little variation in *A. hypogaea* using PCR technologies. However, RAPD marker investigations have indicated that a large amount of genetic variation exists among species of *Arachis* (Halward *et al.*, 1992; Hilu and Stalker, 1995). RAPD markers can be used to identify species and accessions within *Arachis*, and Hilu and Stalker (1995) concluded that *A. duranensis* is the donor of the A genome of *A. hypogaea.* Garcia (1995) used RAPDs to add markers to the RFLP map of Halward *et al.* (1993), and he found colliniarity between RAPDs and RFLPs. Garcia (1995) also investigated genetic variation among plants derived from the cytological pathway of selfing hexaploids and backcrossing at each respective generation after selfing. During each selfing generation he found that increasing numbers of molecular markers were lost, likely resulting from meiotic irregularities and subsequent random loss of genes. However, in a tetraploid population derived by selfing hexaploids, Garcia *et al.* (1995) reported introgression of *A. cardenasii* (A genome species) genes into *A. hypogaea* in 10 of the 11 linkage groups on the diploid molecular map. This indicates that introgression can occur from diploid species to the cultivated species and that the genomes of *A. hypogaea* are very similar.

To add greater specificity to PCR techniques, Paran and Michelmore (1993) developed sequence characterized amplified regions (SCARs) from PCR markers. A SCAR is a genomic DNA fragment at a single genetically defined

locus that is identified by PCR amplification using a pair of specific oligonucleotide primers. SCARs are superior to RAPD markers because they are less sensitive to reaction conditions. They also can be used as codominant genetic markers. Like RAPDs, SCARs do not require radioactivity, and dominant SCARs may be used as a quick plus/minus assay for a particular product.

Garcia *et al.* (1996) used RAPD and SCAR technology to map two dominant genes conferring resistance to the root knot nematode [*Meloidogyne arenaria* (Neal) Chitwood Race 1] in peanut. In their study, the cultivated peanut was crossed with a tetraploid breeding line derived from a resistant species (*A. cardenasii*). Bulked segregant analysis was used to find RAPD markers common to both resistant progeny and *A. cardenasii*. One marker (Z3/265) was closely linked with *M. arenaria* resistance and subsequently mapped to an area known to contain *A. cardenasii* introgression (Garcia *et al.*, 1995). This fragment was cloned to make SCAR and RFLP probes and linkages were subsequently confirmed (Garcia *et al.*, 1996). Burow *et al.* (1996) also reported an RFLP marker (R239) linked to root-knot nematode resistance genes which were derived from tetraploid plants of the hybrid Florunner x 4*x* [*A. batizocoi* (*A. cardenasii* x *A. diogoi*)] by using bulk segregant analyses. The R239 marker for nematode resistance maps to the same linkage group on both the diploid and tetraploid molecular maps. However, Garcia (1995) concluded that the genes in the two crosses are likely different.

Amplified Fragment Length Polymorphisms (AFLPs) are based on PCR-based techniques that combine the benefits of RFLPs and RAPDs (Vos *et al.*, 1995). Like RAPDs, no prior knowledge of nucleotide sequence is required but, unlike RAPDs, this procedure may result in 100 or more fragments per DNA sample. Thus, the method increases the possible number of loci screened about tenfold over RAPD technologies. The first step in AFLP methodology consists of DNA restriction and ligation of oligonucleotide adapters. The restriction is accomplished by digesting the DNA with a frequent cutter (e.g., *Eco*RI) and a rare cutter (e.g., M*se*I). This results in three fragment types such that the M*se*I-M*se*I and *Eco*RI-*Eco*RI fragments are very short and are usually negligible in the reaction, and the M*se*I-*Eco*RI fragments are the only ones amplified. These fragments are 'polar'--that is, the ends are different, and the fragments are extended by ligation with two oligonucleotide primers (one for each end) of which one is labeled. After PCR amplification, fragments are run on a polyacrilamide gel and analyzed. The procedure can be useful for studying complex genomes (i.e., in plants); and by subjecting DNA to PCR with a single selective unlabeled nucleotide, which reduces the number of fragments, background smears on gels are usually reduced.

He and Prakash (1997) were the first investigators to report applications of AFLP technologies in peanut. They used 28 primer pairs to generate 111 AFLP markers in *A. hypogaea*. Their results indicated that about 3% of the primers used for DNA amplification were polymorphic. However, other studies conducted with cultivated peanut have shown less variation (Mila and Stalker, unpubl. data).

Simple Sequence Repeats (SSR) Markers have been reported as being more variable than other marker systems. Southern blots of DNA are digested with restriction enzymes and probed with a number of synthetic oligomers composed of simple nucleotide repeats. These markers are more variable then RFLPs or RAPDs in many species (Edwards *et al.*, 1991; Nanda *et al.*, 1991) and are codominant and easily detected from relatively little amounts of DNA after PCR amplification. Hopkins *et al.* (1999) reported six polymorphic SSRs in *A. hypogaea* with the number of fragments amplified per SSR ranging from two to 14, and differentiated 15 of 19 accessions of cultivated peanut. About 150 SSR polymorphic markers have been identified in diploid species of *Arachis* (Hopkins, pers. commun.).

The Abundance of Morphological Variation within *A. hypogaea* versus the apparent lack of detectable polymorphisms when molecular technologies are applied has been observed in other species--for example in tomato (*Lycopersicon* spp.) (Miller and Tanksley, 1990), melons (*Cucumis* spp.) (Shattuck-Eidens *et al.*, 1990), soybean (*Glycine* spp.) (Keim *et al.*, 1990), and common bean (*Phaseolus* spp.) (Gepts, 1991). Morphological traits often are altered by one or a few major genes and intense selection pressure under cultivation results in diversification. Conversely, Gepts (1991) concluded that variation for biochemical and molecular markers, which are not subject to direct selection, often decreases during domestication. In peanut, genetic studies indicated that a large number of morphological traits are conditioned by a few genes, with expression influenced by the action of modifiers and epistatic interactions among loci (Wynne and Halward, 1989). Intense selection for a few morphological traits after polyploidization and then isolation from other *Arachis* species could explain the apparent lack of variation at the molecular level in *A. hypogaea* (Williams, 1996). Gepts (1991) observed a similar pattern of little molecular variation in the common bean (*P. vulgaris* L.).

A Case Study in Peanut Breeding. Data for molecular analyses of plants is relatively easy to collect, but observing meaningful populations to solve plant breeding problems often is complex and difficult. Much of the problem is acquiring good segregation data for the desired trait(s). The following experiment was initiated with the objective of associating molecular markers with early leaf spot (caused by *Cercospora arachidicola* Hori) resistance, and will illustrate some of the difficulties in choosing parents under field conditions where meaningful selection can occur. In addition, recently acquired information about molecular marker associations with morphological and resistance traits will be presented.

Materials and Methods

Germplasm and Field Ratings. Early and late leaf spot-resistant and susceptible *A. hypogaea* lines and interspecific hybrids were evaluated in the field in two-row plots with 90-cm by 4.6-m spacing (Table 1). Five randomly selected leaves from each of 40 lines were detached from the third or fourth node of branches, and petioles were inserted into trays

Table 1. *Arachis hypogaea* and interspecific hybrids used in leaf spot evaluations.

Entry	Identification	Selection	Leaf spot reaction[a]
1	NC 6//NC 3033/NC GP WS 1b	11	CA highly resistant
2	NC 6//NC 3033/NC GP WS 1	14	CA highly resistant
3	NC 6//NC 3033/NC GP WS 1	15	CA highly resistant
4	NC 6//NC 3033/NC GP WS 1	17	CA highly resistant
5	NC 6//PI 270806/NC GP WS 4	46	CA highly resistant
6	NC 6//PI 270806/NC GP WS 4	48	CA highly resistant
7	NC 6//PI 270806/NC GP WS 4	51	CA highly resistant
8	NC 6//PI 270806/NC GP WS 4	52	CA highly resistant
9	NC 6//PI 270806/NC GP WS 4	53	CA highly resistant
10	NC 5//PI 270806/NC GP WS 4	20	CA highly resistant
11	NC 5//PI 270806/NC GP WS 4	25	CA highly resistant
12	NC GP WS 1		CA resistant
13	NC GP WS 2		CA resistant
14	NC GP WS 3		CA resistant
15	NC GP WS 4		CA resistant
16	PI 270806		CA resistant
17	PI 109839		CA resistant
18	FESR-B2-6		CA resistant
19	NC 3033		CA resistant
20	PI 261942/GKP 10017 - CS22		CA susceptible
26	PI 261942/GKP 10017 - CS26		CP resistant
27	PI 261942/GKP 10017 - CS39		CP resistant
28	PI 196631		CP resistant
29	PI 215696		CP resistant
30	PI 215724		CP resistant
31	PI 261942/GKP 10017 - CS33		CP resistant
32	PI 261942/GKP 10017 - CS62		CP resistant
33	Southern Runner		CP resistant
34	TMV-2		CA, CP susceptible
35	PI 261942/GKP 10017 - IC12		CP susceptible
36	PI 261942/GKP 10017 - CS2		CP susceptible
37	PI 261942		CA,CP susceptible
38	NC 5/PI 270806/NC GP WS 4		CA, CP susceptible
39	NC6		CA, CP moderately susceptible
40	NC7		CA, CP susceptible

[a]Accumulation of data from field tests from N.C. State Univ. and the Int. Crops Research Inst. for the Semi-Arid Tropics (H. T. Stalker, unpubl. data; J. P. Moss, pers. commun.); CA = *Cercospora arachidicola*; CP = *Cercosporidium personatum*.

[b]NC GP WS lines are *A. hypogaea* (PI 261942) x *A. cardenasii* (GKP 10017) 40-chromosome selections for *C. arachidicola* resistance.

of sand in a randomized complete block design and kept in a humidity chamber (Foster *et al.*, 1980). Entries were inoculated with 35,000 spores/mL *C. arachidicola* inoculum. Beginning 14 d after inoculation leaves were scored daily for days to the first lesion and numbers of lesions and also rated for severity of infections (where 1 = no infection to 9 = dead), lesion size, and sporulation at 28 d. The data were subjected to analysis of variance using PROC GLM in SAS (SAS Inst., 1985). Data were transformed by taking the square root of each score for rating and lesion number and analyzed by least significant differences (Steel *et al.*, 1997).

Crosses were made during the summer of 1996 using NC 7 and PI 261942 as females and 20 lines having either early leaf spot or late leaf spot (*Cercosporidium personatum* Berk. et Curt.) resistance. Plants were grown in the field and F_2 seeds could only be harvested in sufficient quantities from crosses involving NC 7, and seeds from 12 crosses were planted in a leaf spot nursery for evaluation. Individual plants were tagged for ratings and two leaves per plant were collected on 27 Aug. (hereafter called "early rating") from the third or fourth node of a randomly selected secondary branches and from a leaf that was considered to be the most diseased leaf. These leaves were viewed under a stereoscope to collect data for the number of lesions per leaf, number of lesions sporulating, percentage sporulating lesions, and diameter of the largest lesion. Individual field plants were rated for leaf spot using the 1 = no disease to 9 = dead plant scale; percentage defoliation was measured by counting the missing leaves on one previously chosen lateral branch, excluding branches from which leaves or cuttings had been taken during the growing season. The same procedure was repeated on 15 Oct., and these ratings were termed "late rating."

Ratings also were made for Cylindrocladium black rot (CBR), caused by *Cylindrocladium crotalariae* (Loos) Bell and Sobers, (disease = 1, no disease = 0); leafhopper (*Empoasca fabae* Harris) damage (1 = susceptible to 4 = resistant); and southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber) (1 = susceptible to 4 = resistant). Plant size (small = 1, medium = 2, large = 3) and color (light green = 1, green = 2, dark green = 3) were recorded. Plants were harvested individually and average pod length, average pod width, average seed length and average seed width were calculated for each plant after measuring 10 pods and 10 seeds. Means and ranges for each trait in each of the 12 populations were calculated using PROC SUMMARY in SAS (SAS Inst., 1985), and populations were compared using an approximation to Student's t test.

Cuttings were taken from each plant, rooted, and potted in the greenhouse into 10.2-cm pots containing soil. DNA was extracted from all plants in the two populations that segregated for disease and morphological traits sufficiently to perform segregation analyses (NC 7 x PI 109839 and NC 7 x NC GP WS 1). Bulks were established for each trait by mixing the DNA from the five most resistant and five most susceptible progeny (or the extremes in the case of morphological traits) for each variable measured.

DNA Isolation. DNA was extracted from parental and hybrid plants by a modification of the procedure described by Kochert *et al.* (1991). Fresh leaf material was dipped in liquid N_2 and stored at -32 C. The frozen leaves were homogenized by blending in 100 mL of ice-cold DNA extraction buffer. The mixture was filtered through two layers of cheesecloth and one layer of Miracloth and then centrifuged in a GSA rotor at 2000 rpm for 15 min at 4 C. The supernatant was discarded and then 5 mL of nuclei lysis buffer, 5 mL DNA extraction buffer, and 2 mL of 5% Sarkosyl were added to each sample. The samples were incubated at 60 C for 15 min and then 15 mL of chloroform:isoamyl was added. The samples were mixed for 15 min to form an emulsion and then centrifuged in a S34 rotor at 2000 rpm for 15 min. The aqueous phase was added to 2 x volume of ice-cold 95% TE at 60 C. Each sample was centrifuged in a microcentrifuge for 10 min and transferred to a new tube. The DNA was quantified in a Spectronic 1201spectrophotometer. The amount of DNA was determined by taking the difference between the 320 and 260 NM reading and multiplying the dilution factor of five (10 µL of sample in 990 µL H_20). The purity was tested by the ratio of 260/280 NM reading.

RAPD reactions contained 5 µL genomic DNA and were

completed in 96 well plates as described by Halward *et al.* (1992) and photographs were analyzed using white instant sheet film (Polaplan 57). Plants from each line were screened with 293 primers from Operon Technologies, Inc. (Alameda, CA) and 56 primers from the Univ. of British Columbia (Vancouver, Canada) in an attempt to associate markers with resistance. Bulk segregant analyses (Michelmore *et al.*, 1991) was applied to each segregating population. Bulks were screened first with 14 primers found to be polymorphic between NC GP WS 1 and *A. hypogaea,* and subsequently with an additional 223 Operon primers of which 33 were polymorphic between parents. Results were viewed using a Stratagene Eagle Eye™ II (Stratagene, La Jolla, CA). Nineteen primers were found with polymorphisms between bulks, and these primers were used to screen all F_2 plants in the populations in which they were polymorphic. Regression analysis was completed using SAS PROC GLM to determine linkage of markers to traits (SAS Inst., 1985). Linkage analysis was completed using the Mapmaker/EXP program to group markers into linkage groups.

Results and Discussion

Forty entries were evaluated in the field for variation to leaf spot resistance, and significant differences were observed (data not presented). Several 40-chromosome hybrid derivatives were highly resistant, other lines were moderately resistant, and selected checks were highly susceptible to *C. arachidicola.* When 20 moderate to highly resistant lines of *A. hypogaea* introductions and interspecific hybrid derivatives were used as parents in crossing programs with NC 7 and PI 261942, many of the hybrids were difficult to obtain. Further, PI 261942 crosses did not produce sufficient numbers of F_2 progenies to evaluate segregation for disease or other morphological traits. Thus, only F_2s from NC 7 hybrids were grown in the field to evaluate resistance to leaf spots; and only progenies from those crosses with relatively large numbers of progenies. DNA was extracted from more than 1800 greenhouse-grown plants of 12 lines.

Parental Lines were evaluated for leaf spot resistance in the field and subjected to molecular analysis (data not shown). Relatively little molecular variation was observed among the 40 entries and no bands were clearly associated with resistance across all lines. However, *A. cardenasii* had many banding patterns different from *A. hypogaea.* Fourteen primers (Operon primers AD 1, AF 6, AH 6, AM 3, AM 11, AM 20, D 10, G 8, G 19, J 19, N 3, O 7, Q 4, and R 4) were polymorphic between the susceptible *A. hypogaea* cultivar and the leaf spot-resistant interspecific hybrid NC GP WS 1.

Hybrid Progenies mostly had very little disease in the field, and only the two crosses NC 7 x PI 109839 and NC 7 x NC GP WS 1 (which contained *A. cardenasii* in its pedigree) were analyzed for segregation of morphological traits, disease and insect resistance, and molecular marker variation. The two populations were significantly different ($P \leq 0.05$) for the following traits: leaf spot rating, sporulation rating on the fourth leaf, early defoliation, CBR resistance, southern corn rootworm resistance, size, color, leafhopper resistance, average pod length, average pod width, average seed length, and average seed width. While the populations were not significantly different for many components of resistance to leaf spot, the NC 7 x NC GP WS 1 population had fewer lesions, lower percentage sporulation, lower sporulation ratings, smaller lesion diameters, and less early defoliation, CBR infection, and were larger plants. The NC 7 x PI 109839 population had lower ratings, less southern corn rootworm infection, less defoliation, less leafhopper damage and darker foliage color.

Within each population, average pod length, average pod width, average seed length, and average seed width were significantly ($P \leq 0.0001$) correlated. The components of *C. arachidicola* resistance (average lesion number, percentage sporulation, sporulation rating, and lesion diameter) had different correlation coefficients in each population. For the NC 7 x PI 109839 population, early rating was significantly correlated ($P \leq 0.05$) with early defoliation, late rating, and late defoliation; however, early defoliation was not correlated with late rating, late defoliation, or color. In this population, color was negatively correlated with defoliation, but not with early rating or early defoliation. Thus, the lighter plants were more defoliated, but the remaining leaves were not necessarily more diseased.

The NC 7 x NC GP WS 1 population was polymorphic for six RAPD primers (Operon primers AD 1, AI 11, AI 19, AJ 19, AK 20, and AN 15). Associations in this population were established between molecular makers and the following traits via linear regression analysis: sporulation rating on the fourth leaf, lesion diameter on the fourth leaf, southern corn root worm damage, early defoliation count, leaf spot rating, average seed length, plant color, early leaf spot rating, lesion diameter on the worst leaf, and harvest defoliation (Table 2).

The NC 7 x PI 109839 population was polymorphic for the three Operon primers AM 11, AN 15 and Q 6. Four marker bands were observed, including two markers (AN 15 and AM 1101) that mapped 28.7 cM apart, and AM 1102 and Q 6 that were not linked. Thus, three areas of the genome were marked in this population. For this reason, simple models were used in the linear regression analysis of traits and markers. The percentage of the variation explained by these models for respective vari-

Table 2. Models for the F_2 RAPD screening for the NC 7 x NC GP WS 1 population.

Trait evaluated	Primers	R^2	CV	P
Sporulation rating (4th leaf)	AD 1	0.1022	142.0	0.034
Lesion diameter (4th leaf)	AD 1	0.1963	55.1	0.003
South. corn rootworm resist.	AJ 19	0.0762	59.2	0.048
Early defoliation	AN 15	0.0840	382.7	0.043
Late rating	AK 20	0.1081	37.6	0.019
Average seed length	AN 15	0.0540	15.3	0.082
Plant color	AI 11	0.1833	37.8	0.001
Early rating	AI 11	0.0881	28.0	0.038
Lesion diam. (worst leaf)	AD 1	0.2359	49.6	0.001
Lesion diam. (worst leaf)	AD 1, AN 15	0.2835	48.7	0.001
Lesion diam. (worst leaf)	AD 1, AN 15, AJ 19	0.3468	48.5	0.001
Defoliation at harvest	AK 20, AJ 19	0.1746	52.2	0.013

ables was less than 9%. Only two traits were significantly correlated to RAPD primers. Cylindrocladium black rot resistance was associated with AM 1101 ($P \leq 0.01$) and sporulation rating of the worst diseased leaf was associated with AM 1102 ($P \leq 0.05$) (Table 3).

Table 3. Models for the F2 RAPD screening for the NC 7 x PI 109839 population.

Trait evaluated	Primer	R^2	CV	P
Leafhopper resistance	AN 15	0.038	59.00	0.113
Sporulation rating (worst leaf)	AM 1102	0.060	79.60	0.044
Early defoliation	AM 1102	0.039	65.30	0.103
Defoliation at harvest	Q6	0.043	61.60	0.095
Cylindrocladium black rot resistance	AM 1101	0.100	152.60	0.009

It is theorized that the two resistant parents have different genes related to individual components of resistance because the NC GP WS 1 contained *A. cardenasii* in its pedigree and the other cross was between two *A. hypogaea* genotypes. The two populations differed in their average response to leaf spot and other characteristics. Early defoliation was not correlated with defoliation at the time of harvest, which indicates that disease progress was not linear. This may also indicate that evaluating defoliation at the time of harvest may not accurately reflect the defoliation during the growing season for genetically diverse peanut lines.

The lack of molecular polymorphism in cultivated peanuts made identification of markers difficult. A relatively small amount of data was produced from an intensive screening of primers where only eight of 572 RAPD primers showed polymorphisms in the populations. The NC 7 x NC GP WS 1 population had six unlinked markers, and the NC 7 x PI 109839 cross had only one linkage group with two markers and two unlinked markers.

Markers associated with resistance and other traits were found for both populations. This is the first time molecular markers have been associated with resistance genes in an *A. hypogaea* x *A. hypogaea* cross. The models developed for gene association in both crosses have low R^2s and relatively high CVs. Genetic models indicated that up to 35% of the variation was attributable to one area of the genome for each trait observed. This indicates that these markers are either near a single gene with a large effect or that a complex of genes with small individual effects are linked. Earlier work with segregating peanut populations with wild species in their pedigree showed that gene introgression occurred in blocks as opposed to randomly throughout the genome (Garcia *et al.*, 1995).

This study indicated that lines with different pedigrees contain different sources of resistance genes and components of leaf spot resistance may be inherited separately. The inheritance of components of leaf spot resistance is complex and may involve multiple types of epistasis. Markers can be associated with resistance and agronomic traits in populations of *A. hypogaea* and in interspecific crosses interspecific crosses with *A. cardenasii* and their pedigree. There is little molecular variation in *A. hypogaea* that can be identified by molecular marker analyses, but markers can be associated with agronomic traits. Molecular marker technology is still in its infancy, and this technology holds great promise for increasing breeding efficiency in peanut.

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