MOLECULAR AND FIELD ANALYSIS OF NEMATODE RESISTANCE IN PEANUT *(Arachis hypogaea* L.*)*.

By

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Root-knot nematodes (*Meloidogyne arenaria*) are the most important nematode pathogens of cultivated peanut (*Arachis hypogaea* L.). Following interspecific hybridization using wild peanut species and backcrossing with the cultivar Florunner, the root-knot nematode resistant cultivars COAN and NemaTAM were released by Texas A&M University. The first objective of this study was to identify differentially expressed genes in the root-knot nematode resistant cultivar NemaTAM and the root-knot nematode susceptible cultivar Florunner following nematode inoculation. Using suppression subtractive hybridization (SSH), a cDNA library was constructed enriched with differentially expressed sequences from root-knot nematode challenged root tissues of NemaTAM and Florunner. This work resulted in the identification of 140 NemaTAM-specific clones and 123 Florunner-specific clones. Gene ontology studies revealed that a large proportion of the NemaTAM-specific sequences encoded pathogen defense proteins (PR proteins) and detoxification /oxidative stress proteins, while the Florunner-specific sequences were associated with genes encoding non-pathogenesis related proteins. This work provides the foundation for further identification and study of genes involved in the molecular defense mechanism of root-knot nematode resistance in peanut.

The second objective was to incorporate the root-knot nematode resistance found in COAN into Florida breeding lines that were high in oleic acid using molecular markers. Crosses involving COAN and susceptible genotypes were made and subsequent generations were screened with both a sequence characterized amplified region (SCAR) marker (Z3/265) and a restriction fragment length polymorphism (RFLP) marker (R2430E). Forty-five of the 50 lines tested (90%) were positive for both molecular markers, and among these positive lines, 88% displayed field resistance to *Meloidogyne arenaria* indicating successful implementation of marker-assisted selection.

The final objective was to clone and characterize peanut resistance genes (*R*-genes) from NemaTAM that may contribute to this cultivar's resistance to the root-knot nematode. Degenerate PCR primers were used to amplify the conserved nucleotide binding site (NBS) encoding regions of *R*-genes from NemaTAM. Analysis of 145 genomic clones revealed that 26% of the sequences had homology to genes belonging to the section *Arachis*, another 18% to genes found in other legumes, and 41% to genes found in other plants. Of the remaining clones, 15% had sequences related to genes found in microorganisms. Interestingly, one NemaTAM clone shared conserved motifs found in the well-characterized tomato root-knot nematode resistance gene, *Mi*. This cloned peanut *R*-gene was further characterized.

CHAPTER 1
INTRODUCTION AND RATIONALE

Cultivated peanut (*Arachis hypogaea* L.) is an internationally prominent crop for both human consumption and oil seed production. Peanut ranks fifth in the world among oil seed crops (Marcio et. al., 2004). World-wide it is grown on 26.4 million hectares with a production of 36 million tons (FAO, 2004). Peanut is currently being cultivated in over 110 countries primarily between 40^{o} N and 40^{o} S for edible oil, high seed protein, vitamins, and confectionary use. In the United States, it is grown in on approximately 0.6 million hectares with total production of approximately 2 million metric tons annually (http://www.fas.usda.gov/psd/complete_tables/OIL-table11-186.htm). Additionally, per person consumption of peanut products is about six pounds annually (Beyer et al., 2001). It is estimated that the peanut crop contributes approximately $1 billion to the U.S. economy each year (Sasser and Freckman, 1987).

On a global scale, for all crops, losses attributed to plant-parasitic nematodes are estimated to be $78 billion annually (Sasser and Freckman, 1987). Specifically for peanut, economic losses due to root-knot nematodes (*Meloidogyne* species) have been significant in many production regions (Florida, Georgia, Alabama, Texas and North Carolina) with estimated annual monetary losses of $102 million. Also, a 50% decrease in peanut yield potential has been observed due to *M. arenaria* alone (Dickson and De Waele, 2005). Root-knot nematode infection of peanut plants results in heavy galling of pegs, pods and roots. This in turn reduces peanut quality and yield (Rodriguez-Kabana et al., 1986).

Cultivated peanut is susceptible to various pathogens, including root-knot nematodes, due to its genetic uniformity. Conversely, wild *Arachis* species are highly polymorphic and represent a tremendous reservoir of resistance genes. Therefore, several efforts have been made to

introgress valuable genes from wild species into cultivated peanut. One such effort led to the development of two root-knot nematode resistant cultivars, COAN (Simpson and Starr, 2001) and NemaTAM (Simpson et al., 2003). Because all of the wild species involved in the development of COAN and NemaTAM were found to be susceptible to root-knot nematodes, except for *Arachis cardenasii*, it was hypothesized that root-knot nematode resistance was due to introgression of resistance gene(s) from *A. cardenasii* (Garcia et al., 1996). To date, however, there has been a lack of information regarding a comparative response, at the molecular level, of root-knot nematode resistant and susceptible peanut cultivars to root-knot nematode challenge. Consequently, the first objective of the present study was to investigate the molecular mechanisms that may be functioning in the root-knot nematode resistant cultivar NemaTAM, and the root-knot nematode susceptible cultivar Florunner.

Additionally, peanut cultivars that combine the desirable high oleic fatty acid trait (Norden et al., 1987) with resistance to root-knot nematodes are unavailable in the southeastern U. S. Thus, the second objective of this research was to incorporate high oleic fatty acid seed oil and root-knot nematode resistance into the Florida peanut germplasm. Initially, peanut seeds that were classified according to their oleic acid content (Moore and Knauft, 1989) were subsequently screened for root-knot nematode resistance by employing marker-assisted selection using a previously identified SCAR marker, Z3/265 (Garcia et al., 1996), and an RFLP marker, R2430E (Church et al., 2000). RFLP analysis is more labor intensive and costly than SCAR analysis. SCAR marker analysis is simple, rapid and cost effective for screening a large number of breeding populations. However, the particular SCAR marker (Z3/265) used in this analysis, which is derived from *A. cardenasii*, is 10 - 14 cM from the root-knot nematode resistance gene(s) introgressed into cultivated peanut (Garcia et al, 1996). Therefore, more tightly-linked

flanking markers would be desirable to provide plant breeders with more precision when selecting for root-knot nematode resistant plants within segregating populations. Hence, the third objective of this research was to identify resistance genes in NemaTAM that may be tightly-linked to the root-knot nematode resistance trait.

CHAPTER 2
REVIEW OF LITERATURE

Molecular Markers and Linkage Maps in Peanut

Marker-assisted selection (MAS) is a technology that can improve the efficiency of conventional plant breeding. Molecular markers are particularly advantageous for traits where traditional field selection is difficult, expensive, or lacks precision (Crouch, 2001). The most essential requirements for developing MAS breeding programs include: (1) polymorphisms in germplasm with useful characteristics, (2) the identification of flanking markers closely linked to the gene/quantitative trait loci, (3) a simple polymerase chain reaction (PCR)-based marker technology for cost effective screening of large breeding populations, and (4) proper screening techniques for phenotyping of mapping populations (Dwivedi et al., 2003). Molecular markers offer several advantages over morphological markers such as phenotypic neutrality, independence from environmental conditions, independence of detection related to plant age, and segregation at a 1:1 ratio between marker segregation and the genetic constitution of the individual (Dwivedi et al., 2003).

One of the main advantages of using molecular markers is the time gained to introgress resistance genes into cultivars (Tanksley et al., 1989; Melchinger, 1990). The use of DNA markers allows selection of the resistant offspring with the lowest amounts of the donor genome in every generation (Tanksley et al., 1989). Molecular markers are especially useful in root-knot nematode resistance breeding because they: (1) minimize the need for screening large numbers of individuals once the marker-trait relationship is established, (2) eliminate the need for destructive sampling of plants for root-knot nematode evaluations, (3) ease the identification and transfer of recessive genes, (4) reduce linkage drag by allowing selection of segregating

populations with minimum donor DNA, and (5) facilitate map-based cloning of root-knot nematode resistance genes.

Only recently have molecular techniques been employed in peanut to interpret its genome organization. Although great variation for morphological and physiological traits has been observed in cultivated peanut, little polymorphism has been detected at the molecular level relative to other crops (Kochert et al., 1991). However, extensive variation has been observed in wild *Arachis* species (Kochert et al., 1991; Halward et al., 1991, 1993; Paik-Ro et al., 1992; Stalker et al., 1994; He and Prakash, 1997; Hopkins et al., 1999; Subramanian et al., 2000).

Both RFLP- and PCR-based markers have been used to assess polymorphic variation in cultivated peanut and wild *Arachis* species. Halward et al. (1991) employed RAPDs and RFLPs to study genetic variation among wild *Arachis* species and unadapted germplasm resources of cultivated peanut from South America, Africa, and China. They observed abundant polymorphism among wild *Arachis* species, but very little within the cultivated peanut germplasm. Using RAPDs, Lanham et al. (1992) detected 49 polymorphic loci between cultivated peanut (TMV 2) and a synthetic amphiploid $(B \times C)^2$ developed from a cross between *Arachis batizocoi* and *Arachis diogoi*. Of these 49 polymorphic loci, only two were polymorphic in the cultivated peanut germplasm. Paik-Ro et al. (1992) studied RFLP variation in six peanut species within the section *Arachis* (*A. monticola, A. batizocoi, A. cardenasii, A. glandulifera, A. duranensis* and *A. hypogaea*). Using 23 random genomic and seed cDNA probes, they reported that most of the genomic probes and more than half of the seed cDNA probes showed polymorphism among the accessions of the tetraploid species. However, no polymorphism could be detected within or between *A. hypogaea, A. monticola,* and interspecific derivatives. Halward et al. (1993), using primers of arbitrary sequence, studied polymorphic variation in two peanut

cultivars, 25 unadapted *A. hypogaea* germplasm, the wild allotetraploid progenitors of cultivated peanut *A. monticola*, *A. glabrata* (another tetraploid species) from section *A. rhizomatosae*, and 29 diploid wild species of *Arachis*. They observed abundant polymorphism among wild relatives, but did not find any polymorphism in the cultivated peanut germplasm.

Bhagwat et al. (1997) used 12 primers and studied random amplified polymorphic DNA (RAPD) profiles between peanut cultivar Spanish Improved and its mutants obtained following X-ray irradiation. They detected 1182 fragments of which 65 were polymorphic. He and Prakash (1997), using and amplified fragment length polymorphism (AFLP) and DNA amplification fingerprinting (DAF), studied DNA polymorphism in six diverse accessions of cultivated peanut from three botanical varieties (*hypogaea*, *fastigiata*, and *aequatoriana*). The analysis detected 111 polymorphic AFLP markers with an average of 6.7 polymorphic bands per primer, while DAF analysis detected 63 polymorphic markers with an average of 3.7 DAF polymorphic bands per primer. Hopkins et al. (1999) identified six polymorphic simple sequence repeat (SSR) primers and detected 10 putative SSR loci in cultivated peanut. Similarly, Dwivedi et al. (2001) studied RAPD profiles of 26 cultivated peanut accessions and detected 41% genetic dissimilarity. Other studies also revealed the presence of some DNA polymorphism in cultivated peanut using SSR (Hopkins et al., 1999) and RAPD analysis (Subramanian et al., 2000).

Halward et al. (1993) were the first to report on a RFLP-based genetic linkage map of peanut. They used both random genomic DNA and cDNA clones from the peanut cultivar GK 7 (subsp. *hypogaea var. hypogaea*). In total, 100 genomic and 300 cDNA clones were evaluated on F_2 populations derived from the interspecific cross between *A. stenosperma* and *A. cardenasii*. Only 15 genomic and 190 cDNA clones revealed polymorphism among the mapping parents, and of these, 132 markers were further used to study segregation. This work resulted in 117

markers that spanned across 11 linkage groups with a total map distance of approximately 1063 cM. Subsequently, Burow et al. (2001) reported the development of a RFLP-based tetraploid genetic linkage map derived from a cross between Florunner and a synthetic amphidiploid {[(*A. batizocoi* K9484 x (*A. cardenasii* GKP 10017 x *A. diogoi* GKP 10602)]^{4X}}. This map consists of 370 RFLP loci mapped over 23 linkage groups with a total map distance of 2210 cM.

Nematode Resistance Introgression Using Molecular Markers

In soybean, PCR-based markers closely linked to alleles at the *Gro1* and *H1* loci, conferring resistance to soybean cyst nematode, have been developed for use in resistance breeding (Niewöhner et al., 1995). For another major pathogen of soybean, the southern root-knot nematode (*Meloidogyne incognita*), two major QTLs conferring resistance have been mapped using RFLP markers (Concibidio, et al., 1997). In barley, two nematode (*Heterodera avenae*) resistance genes *Ha1* and *Ha2* have been mapped to chromosome 5 using molecular markers (Barr et al., 1998).

In peanut, Garcia et al. (1995) closely monitored introgression of *A. cardenasii* chromosome segments into 46 lines derived from a cross between *A. hypogaea* and *A. cardenasii* using 73 RFLP probes and 70 RAPD primers. They reported that *A. cardenasii* introgressed segments represent approximately 360 cM in the diploid peanut genome by using 34 RFLP probes and 45 RAPD primers that detected *A. cardenasii* segments in one or more introgression lines. Subsequently, two SCAR markers linked to root-knot nematode (*Meloidogyne arenaria* race 1) resistance, Z3/265 (Garcia et al., 1996) and RKN440 (Burow et al., 1996), have been developed. Choi et al. (1999) reported that RFLP probes R2430E, S11137E, and R2545E were linked with resistance to root-knot nematodes in BC_5F_2 populations of the cross Florunner (root-knot nematode susceptible cultivar) x TxAG 7 (a parent of the root-knot nematode resistant cultivar COAN). Recently, Chu et al. (2007) reported the identification of a root-knot nematode

resistant dominant marker, 197/909, in crosses involving COAN and various susceptible parents. These studies demonstrated the utility of molecular markers to tag and subsequently enhance the introgression of desirable traits from wild *Arachis* into cultivated peanut.

Management of *Meloidogyne arenaria* Race 1 in Peanut

Cultivated peanut is infected by several species of root-knot nematodes, but *Meloidogyne arenaria* (Neal) Chitwood (peanut root-knot nematode) is the most common species infecting peanut (Dickson, 1998). In the United States it is the most important nematode pathogen of peanut in Alabama, South Carolina, Georgia, Texas and Florida (Sturgeon, 1986). Based on the ability to infect and reproduce on peanut, two races: *M. arenaria* race 1 and race 2, were proposed (Sasser, 1982). Of the two races, only race 1 has the ability to infect and reproduce on peanut.

The female of the root-knot nematode is capable of producing up to 2,500 eggs at the infection site. The embryo inside the egg develops into a first-stage juvenile (J1). The J1 molts inside the egg shell to a second stage-juvenile (J2). The J2 hatches, becomes mobile and is the infective stage. The J2 body is slender, tapering to bluntly rounded anterior and sharply pointed posterior end. After three additional molts, an adult is produced. The male body is variable in length and tapers to bluntly rounded ends (Cliff and Hirschmann, 1985). Microscopic studies of several populations of males of *M. arenaria* revealed a low, rounded, posterior sloping head cap, which was nearly as wide as the head region (Eisenback, 1980). The mail tail is short without bursa. Males have slender articulated spicules that protrude from the cloaca, and their gubernaculum is small, slender and crescent-shaped. The female of *M. arenaria* is pear-shaped with a pearly white body and prominent neck, but without a tail protuberance (Dickson, 1998).

Peanut plants that are heavily infected by root-knot nematode show severe stunting, chlorosis and wilting symptoms. Abnormal swellings (galls), appear on the roots, pegs and pods (Dickson, 1998).

Root-knot nematodes in peanut are most commonly managed by crop rotation and the application of nematicides (Abdel-Momen et al., 1998). Because the cash value for peanut is relatively low, crop rotation may be the most profitable way of managing *M. arenaria* race 1 (Dickson and De Waele, 2005). Tropical forage grasses, such as bahiagrass (*Paspalum notatum* Flugge), are reported to be one of the best crops to manage population densities of *M. arenaria* in Florida (Norden et al., 1977; Dickson and Hewlett, 1989). Kokalis-Burelle et al. (2002) reported that peanut followed by two years of switch grass suppressed *M. arenaria* population densities in peanut growing fields. Also, field corn was reported to be marginally effective in suppressing *M. arenaria* population densities (Johnson et al., 1999; Dickson, 1998). Cotton is another crop that can be rotated with peanut since *M. arenaria* does not infect cotton (Sasser and Carter, 1982, Dickson and De Waele, 2005). However, one should not rely solely on crop rotation because some root-knot nematode populations survive the winter without a host, and numerous weeds support nematode reproduction to some extent (Dickson and De Waele, 2005).

In severely infested fields, and when other cultural methods of management do not work, then chemical control by nematicides is the most effective and reliable method of managing root-knot nematodes in peanut (Dickson and De Waele, 2005). Farmers in the southeastern U. S. have widely relied on nematicides to control *M. arenaria* populations (Dunn, 1988; Rodriguez-Kabana and Ivey, 1986; Dickson and Hewlett, 1988). In general, two types of nematicides are available, fumigants and nonfumigants. Until their use was suspended in the 1970s and 1980s, the fumigants 1, 2-dibromo-3-chloropropane (DBCP) and ethylene dibromide (EDB) were

available. They were suspended because of human toxicology and environmental concerns (Dickson and Hewlett, 1988; Dickson and De Waele, 2005). Currently, 1, 3-dichloropropene (1, 3-D) is the only fumigant available on the market for use on peanut. A preplant broadcast application of 1, 3-D at a relatively high rate (84 liters/ha) on *M. arenaria* infested fields consistently increased peanut yields compared to nontreated controls (Dickson and Hewlett, 1988).

Several nonfumigant nematicides such as aldicarb, and oxamyl are currently registered for nematode control in peanut (Dickson and De Waele, 2005). Among these nonfumigant nematicides, aldicarb is the most effective when applied preplant or postplant (Dickson, 1998). A postplant application of aldicarb (10 kg a.i./ha) increased peanut yields significantly over the nontreated control (Dickson and Hewlett, 1989). Although nematicides are one of the best management tactics for controlling *M. arenaria*, their continued use in the future has become uncertain because of their economics and environmental hazards (Chen et al., 1996). The nonfumigants are less reliable when *M. arenaria* population densities are very high (Dickson and Hewlett, 1988).

Biological control of *M. arenaria* is another alternative tactic that is receiving attention (Chen and Dickson, 1998). Worldwide, *Pasteuria* spp., have the ability to infect and suppress a wide variety of nematodes (Sayre and Starr, 1988). By field observations (Bird and Brisbane, 1988; Dickson et al., 1991; Oostendorp et al.,1991) and by using microplot and greenhouse experiments (Channer and Gowen,1988; Chen and Dickson, 1998; Davies et al.,1988), it was demonstrated that *P. penetrans* has the ability to control plant-parasitic nematodes. *Pasteuria penetrans* is one of the most effective biological agents that parasitize *M. arenaria* in peanut fields in Florida and Georgia (Minton and Sayre, 1989; Dickson et al., 1994). *Pasteuria.*

penetrans is an endospore forming obligate bacterial parasite on *Meloidogyne* spp. (Chen et al., 1996; Dickson et al., 1994). Although J2 develop into females, when parasitized by *P. penetrans*, their fecundity is greatly reduced (Bird, 1986). Dickson et al. (1991) observed increased peanut yields over a period of 5 years in fields near Williston, FL that were infested with *M. arenaria* and *P. penetrans*. Chen et al. (1996), using microplot experiments, showed that *P. penetrans* applied at the rate of 10,000 and 100,000 endospores/g in soil infested with *M. arenaria* race 1 had increased peanut pod yields of 94% and 57%, respectively. These results clearly demonstrate the potential of *P. penetrans* to suppress *M. arenaria* race 1 in peanut. Further evaluation of *P. penetrans* revealed that initial endospore infection of J2 during planting is the main suppressive mechanism of *M. arenaria* race 1 in peanut (Chen et al., 1997). Additionally, Cetintas and Dickson (2004) studied the long term effects of continuous growth of peanut (4 years) in bahiagrass, rhizomal peanut, and weed fallow plots on persistence of *P. penetrans*. They found weed fallow plots supported the greatest endospore densities followed by bahiagrass and rhizomal peanut. They further studied the vertical distribution of endospores of *P. penetrans* and found that rain and irrigation water has the potential to move endospores deeper into the soil. However, they found the majority of endospores in the top 0 – 30 cm of soil (Cetintas and Dickson, 2005). The control of *M. arenaria* by *P. penetrans* in peanut fields did not appear to be effective in terms of yield until the third growing season (Dickson, 1998; Weibelzahl-Fulton, 1998).

Due to economic considerations, simplicity, and ease of use for farmers, nematode resistant cultivars are often the best method of managing *M. arenaria* (Taylor, 2003). Baltensperger et al. (1986) first reported resistance to *M. arenaria* in *Arachis glabrata* Benth., however this species is not cross compatible with cultivated peanut. In a later study, Nelson et al.

(1989) reported *M. arenaria* resistance in 21 *Arachis* species and two interspecific hybrids. Although these resistance sources suppress *M. arenaria* population densities by only 40 – 60% (Noe et al., 1992), their yield potentials are equal to that of the susceptible standards in the absence of *M. arenaria* pressure and are superior in the presence of *M. arenaria* pressure (Holbrook et al., 1995).

The Texas A&M University peanut breeding program has given considerable attention toward searching, collecting, introducing, preserving and evaluating wild *Arachis* germplasm. Initially, efforts were mainly directed toward utilization of the wild species for improvement of the cultigens (Simpson, 1991). All attempts to introgress root-knot nematode resistance from *A. cardenasii* and *A. chacoensis* into Tamnut 74 (a Spanish market type) failed due to a lack of fertility. So, further efforts were focused on the development of resistance to *M. arenaria* through a diploid route (Simpson, 1991). An *A. cardenasii* x *A. chacoensis* diploid hybrid was crossed to *A. batizocoi*. The resulting diploid three way hybrid, upon colchicine treatment, produced a tetraploid progeny expressing elevated hybrid vigor, pollen stability (92%), and high fertility. In another attempt, F_1 obtained from a cross between two diploid (AA) wild *Arachis* species, *A. cardenasii* and *A. diogoi,* were crossed with another diploid (BB) wild species *A. batizocoi*. The diploid (AB) F_2, thus obtained, when doubled by colchicine treatment, resulted in the tetraploid interspecific hybrid, TxAG-6, which was then crossed with Florunner to produce another interspecific hybrid designated as TxAG-7. This hybrid, when back crossed five times with Florunner, led to the development of COAN, the first root-knot nematode resistant peanut cultivar (Simpson and Starr, 2001). Two further backcrosses of COAN with Florunner led to NemaTAM (Simpson et al., 2003). Because all of the wild species involved in these crosses were susceptible to root-knot nematodes, except for *A. cardenasii,* it was hypothesized that resistance

in COAN and NemaTAM was due to introgression of segment(s) of DNA from *A. cardenasii* (Garcia et al., 1996; Fig. 2-1). In nematode infested fields both of the *M. arenaria* race 1 resistant cultivars, COAN and NemaTAM, may have greater yield potential than susceptible cultivars (Dickson and De Waele, 2005). However, in fields not infested with root-knot nematodes, the yield potential of COAN is lower than the highest yielding susceptible cultivars (Starr et al., 2002).

Stalker et al. (1994) also focused their efforts to develop resistance to *M. arenaria* using *A. cardenasii* as the source of resistance through a hexaploid route. Several of the breeding lines that were developed had complex segregation patterns, indicating that resistance to *M. arenaria* in *A. cardenasii* is probably conditioned by multiple, dominant, major genes (Starr and Simpson, 1991). Similarly, it was reported that resistance to *M. arenaria* derived from *A. cardenasii* is controlled by more than two genes with major effects (Garcia et al., 1996; Choi et al., 1999). These observations indicate that more than two genes with major effects confer resistance in *A. cardenasii*. However, resistance in the F_2 generation from TxAG6 x *A. hypogaea* and a derived BC3 population segregated in a 3:1 ratio, indicating a single dominant gene (Church et al., 2000). Bendezu and Starr (2003) also reported that resistance to *M. arenaria* in COAN is controlled by a single dominant gene. Therefore, it appears likely that only one gene was introgressed into COAN and subsequently into NemaTAM due to conventional breeding and selection. NemaTAM and Florunner, being nearly isogenic, should differ mainly with respect to root-knot nematode resistance.

Plant *R* Genes, Classes and Conserved Functional Domains

During plant-pathogen interactions, resistance and susceptible reactions are often controlled by the compatibility interaction of plant resistance genes (*R* genes) and their corresponding pathogen-encoded avirulence genes (*Avr* genes). Matched specificity between *R*

genes and *Avr* genes trigger signal transduction pathways leading to further activation of host defense mechanisms (Lamb, 1996). Each plant can have many *R* genes and similarly a pathogen can have many *Avr* genes. One of the most important characteristics of the matched interaction between an *R* gene and an *Avr* gene is the activation of a hypersensitive reaction, during which plant cells surrounding the pathogen infection site undergo programmed cell death (Jones and Dangl, 1996). Other features of a defense response include production of salicylic acid, Ca^{2+} and other ion fluxes, generation of activated oxygen species such as super oxide (Dixon et al., 1994), and synthesis of antimicrobial metabolites and harmful enzymes such as chitinases and glucanases (Dixon and Lamb, 1990; Dixon et al., 1994).

Several *R* genes that confer resistance to a wide spectrum of pathogens, including viruses, bacteria and fungi have been cloned from different plant species. The protein products of most of these *R* genes share common sequence motifs that are involved in signal transduction and protein-protein interactions (Michelmore 1996; Hammond-Kosack and Jones 1997; Martin et al. 2003). Based on their deduced amino acid sequences and the combination of R protein structural domains, *R* genes have been classified into five major classes (Soriano et al., 2005).

The first, and the most frequent class, is comprised of genes that code for proteins containing the nucleotide binding site-leucine-rich repeat (NBS-LRR) domain (Bent et al., 1994; Whitham et al., 1994). The NBS region consists of conserved motifs such as the phosphate-binding P-loop (Kinase motif) with the consensus sequence GxGxxGR(T/S), where x stands for any residue, a hydrophobic domain (sequence GLPLxL) and two additional kinase domains, kinase 2 and kinase 3a (Soriano et al., 2005). The kinase 2 domain is proposed to coordinate the metal ion binding required for phospho-transfer reactions. Similarly, the arginine in the kinase 3a domain interacts with the purine base of ATP in other proteins (Traut, 1994). Because of the

structural similarity of the NBS region with cell death genes in *Caenorhabditis elegans* and humans, it is hypothesized to be involved in hypersensitive (HR) cell death upon recognition of *Avr* genes in plants (Biezen et al., 2002). The LRR region consists of conserved motifs with leucine, and in some cases, proline and asparagine (Soriano et al., 2005). This portion is hypothesized to be involved in protein-protein interactions (Kobe and Deisenhofer, 1994), and to have a pathogen-specific recognition function (Takken and Joosten, 2000).

Proteins belonging to the NBS-LRR class can be further divided into sub-classes depending on their N-termini. These are proteins having a leucine-zipper (LZ) or other coiled-coil sequence (CC) at their N-termini, and those having N-termini referred to as TIR because of their similarity to the *Drosophila* Toll and the mammalian Interleukin 1 receptor (Bent, 1996; Hammond-Kosack and Jones, 1997; Hulbert et al., 2001).

Several *R* genes belonging to the NBS-LRR class have been cloned and characterized in plants. Among these are the tobacco *N* gene conferring resistance to tobacco mosaic virus (TMV) (Whitham et al. 1994), *Arabidopsis RPM1 and RPS2* conferring resistance to the strains of the bacterial blight pathogen *Pseudomonas syringae* and *RPP5* conferring resistance to the fungus *Peronospora parasitica* (Bent et al., 1994; Mindrinos et al., 1994), the *I2C-1* locus in tomato conferring resistance to the tomato vascular wilt fungus *Fusarium oxysporum* f. sp. *lycopersici* race 2 (Ori et al., 1997), the *L6* and *M* genes conferring resistance to flax rust caused by the fungus *Melampsora lini* (Lawrence et al. 1995; Anderson et al. 1997), and the *Xa1* gene conferring resistance to the rice bacterial blight pathogen *Xanthomonas oryzae* (Oshimura et al. 1998).

The second class of *R* genes codes for proteins containing extra-cytoplasmic LRR domains and a C-terminal transmembrane domain (Dixon et al. 1996). This class includes the gene

families, *Cf-2*, *Cf-4, Cf-5* and *Cf-9* of tomato conferring race-specific resistance to the leaf mold pathogen *Cladosporium fulvum* (Hammond-Kosack et al., 1996). These genes confer resistance by specifically recognizing the avr gene products secreted by the fungus *C. fulvum* (Van den Ackerveken et al., 1992; Jones et al., 1994; Dixon et al., 1996).

The third class of *R* genes encodes proteins with a serine/ threonine protein kinase domain with no LRR domain (Martin et al., 1993). This class includes the first race-specific *R* gene, the tomato *Pto* gene that governs resistance to the bacterial speck pathogen *Pseudomonas syringae* pv. *Tomato* (Martin et al., 1993). *Pto* upon recognizing the *avrPto,* codes for a 321-amino acid protein containing a serine/threonine-specific protein kinase domain. *Pto* is capable of phosphorylating additional proteins which then leads to cellular defense responses against *Pseudomonas syringae* pv. *Tomato* (Bogdanove, 2002).

The fourth class of *R* genes code for products that contain both the extra cellular LRR domain and the cytoplasmic protein kinase (Song et al., 1995). This class includes the rice *Xa21* gene that governs resistance to over 30 strains of the bacterial leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae* (Song et al., 1995). This gene product possesses both the LRR feature of the Cf-9 protein and a Pto-like serine/threonine kinase domain. Therefore, it was proposed that the *Xa21* LRR domain must be involved in recognition of extra cellular pathogen *avr*-gene products, and the kinase must be involved in phosphorylating additional proteins that lead to cellular defense responses against *Xanthomonas oryzae* pv. *oryzae* (Hulbert et al., 2001).

The fifth, and final, class includes *R* genes that do not fit into any of the above classes. The best example is the first cloned *R* gene, the maize *Hm1* that encodes a toxin reductase (Johal and Briggs, 1992). This gene confers resistance to *Cochliobolus carbonum* by encoding a NADPH-dependent reductase that inactivates a potent plant toxin produced by the plant upon infection. In

this case, the defense response does not involve specific recognition of an avr-gene product by the Hm1 gene product (Johal and Briggs, 1992).

Several *R* genes against nematodes have been cloned and characterized. Other than the first nematode *R* gene identified, *Hs1pro-1*, all the other nematode *R* genes share structural homology with other plant disease resistance genes (Baker et al., 1997; Cai et al., 1997; Ellis and Jones, 1998; Williamson, 1998). Hs1pro-1 only contains a leucine-rich domain but not a true LRR. Additionally, it has an N-terminal signal sequence that might facilitate cytoplasmic membrane localization (Cai et al., 1997). Most of the other nematode *R* genes code for members of a family characterized by NBS-LRRs (Lagudah et al., 1997; Milligan et al., 1998). For example, the potato cyst nematode resistance gene, *Gpa2*, encodes a product with NBS and LRR domains (Van der Vossen et al., 2000). Similarly, the well characterized tomato nematode resistance Mi protein belongs to NBS-LRR family and has an additional N-terminal region that encodes a leucine zipper domain, which might be involved in protein dimerization (Atkinson et al., 2003). Another tomato nematode resistance gene, *Hero*, codes for a 148 kDa protein that also belongs to the NBS-LRR family with an additional N-terminal leucine zipper domain (Atkinson et al., 2003).

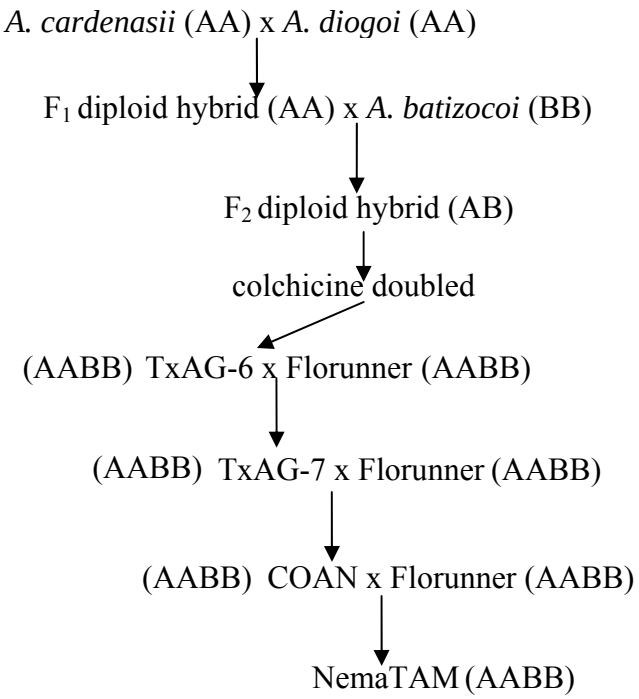

Figure 2-1. Breeding scheme used to develop the root-knot nematode resistant peanut cultivars into COAN and NemaTAM (Burow et al., 1996).

CHAPTER 3
CHARACTERIZATION BY SUPPRESSION SUBTRACTIVE HYBRIDIZATION OF TRANSCRIPTS THAT ARE DIFFERENTIALLY EXPRESSED IN ROOTS OF ROOT-KNOT NEMATODE RESISTANT AND SUSCEPTIBLE CULTIVARS OF PEANUT (*ARACHIS HYPOGAEA* L.)

Introduction

Plant-parasitic nematodes are a serious threat to numerous crops around the world and are responsible for average annual crop losses of as much as $78 billion (Sasser and Freckman, 1987). Monetary losses due to nematodes in cultivated peanut (*Arachis hypogaea* L.) alone are estimated at $102 million (Sasser, 1990). In the United States, economic losses due to root-knot nematodes (*Meloidogyne* species) can be significant in many peanut-producing states (e.g. Alabama, Florida, Georgia, North Carolina, and Texas).

Peanut is parasitized by three major *Meloidogyne* species, viz. *M. javanica*, *M. hapla* and *M. arenaria* and each is capable of severely suppressing yields. Among these root-knot nematodes, *M. hapla* is the least damaging, whereas *M. javanica* and *M. arenaria* are more destructive (Dickson and DeWaele, 2005). For example, when *M. arenaria* is present at damaging levels, that is as low as a single juvenile/100 cm^3 of soil in the case of Florida (McSorley et al.,1992), an approximate 50% decrease in peanut yield potential can be observed (Dickson and DeWaele, 2005). In peanut, two host races of *M. arenaria* have been defined based on their ability (race 1) or inability (race 2) to reproduce on the peanut cultivar Florunner (Sasser, 1954).

Polyploidization has reproductively isolated cultivated tetraploid peanut from its diploid wild relatives that contain a reservoir of resistance genes. The effect of this genetic bottle-neck has been a low level of genetic variability in cultivated peanut and this has resulted in high vulnerability to root-knot nematodes in general, and *M. arenaria* specifically. Although chemical control of root-knot nematodes using nematicides is most effective (Dickson and Hewlett, 1988),

it has limitations due to governmental regulations and environmental concerns (Chu et al., 2007). Consequently, deployment of resistant cultivars has become a more attractive and desirable management tactic (Zijlstra et al., 2000). An effort to incorporate root-knot nematode resistance from the wild species, *A. cardenasii*, into cultivated peanut led to the development of the interspecific hybrids, TxAG-6 and TxAG-7 (Simpson et al., 1993; Starr et al., 1995). A backcross breeding program was then used to incorporate the nematode resistance found in these hybrids into peanut breeding populations, which ultimately lead to the release of COAN, the first peanut cultivar with resistance to *M. arenaria* (Simpson and Starr, 2001). Further back-crossing of COAN with Florunner led to the release of the near isogenic cultivar, NemaTAM (Simpson et al., 2003).

It was first reported that the resistance to *M. arenaria* derived from *A. cardenasii* is controlled by two genes with major effects (Garcia et al., 1996; Choi et al., 1999). Garcia et al. (1996) reported that two dominant genes, designated as *Mae* and *Mag*, may restrict egg number and galling, respectively, in a segregating F_2 population derived from the cross of 4x (*Arachis hypogaea* X *Arachis cardenasii*)-GA 6 and PI 261942. However, Bendezu and Starr (2003) reported that resistance to *M. arenaria* race 1 in COAN is controlled by a single dominant gene. They also observed that most of the second-stage juveniles (J2) found in the roots of COAN were restricted to the cortical tissue with only one out of 90 J2 observed being associated with vascular tissues. However, greater than 70% of the J2 were associated with vascular tissues in Florunner (Bendezu and Starr, 2003). They also reported that root-knot nematode resistance in COAN might be due to constitutive factors in the roots. However, there have been no gene characterization studies involving root-knot nematode infection in peanut. Consequently, the objective of the present study was to investigate the molecular mechanisms that may be

functioning in the root-knot nematode resistance and susceptible responses in peanut as exhibited by NemaTAM and Florunner, respectively. Suppression subtractive hybridization (SSH) was used to identify differentially expressed ESTs in roots of both NemaTAM and Florunner following exposure to root-knot nematodes. Results obtained lead to a hypothesis for a root-knot nematode resistance mechanism that may be operating in NemaTAM.

Materials and Methods

Plant Materials and Root-knot Nematode Inoculation

Individual plants of NemaTAM and Florunner were grown in clay pots containing pasteurized soil (Arredondo and Sparr type; sand 95%, silt 3%, clay 2% and organic matter 1.5%; pH 6.5) maintained in a greenhouse at 26 - 32 ^{o}C, where they were watered daily and fertilized once a week with N-P-K (24-8-16; Miracle-Gro, Marysville, OH). Ten days after planting, each pot was inoculated with a suspension of 5000 eggs of *M. arenaria* race 1 that had been cultured on tomato (*Solanum esculentum* Mill. cv. Rutgers). Eggs were pipetted into three holes (0.6-cm diameter x 4-cm depth) made close to the roots, but distributed at an equal distance from the base of the plant. Root-knot nematode inoculum was prepared using the NaOCl method as described by Hussey and Barker (1973). Whole roots from five plants per cultivar were collected for RNA extraction at five time points: 0, 12, 24, 48, and 72 h after inoculation.

RNA Extraction

Total RNA was isolated from the root samples described above using the Guanidine–HCl method of Logemann et al. (1987) with slight modification. Roots (3 g) were ground in liquid nitrogen followed by the addition of 15 ml of 6 M Guanidine buffer and 15 ml of phenol/chloroform/isoamyl alcohol (PCI; 25:24:1). The mixture was shaken for 20 min and centrifuged for 20 min (20, 000 x g) at 4 ^{o}C to separate the phases. The supernatant was gently removed and 1.5 vol of absolute ethanol and 0.3 vol of 1M acetic acid (pre-cooled at 4 ^{o}C) were

added, and the RNA was precipitated overnight at –20 ^{o}C. The pellet obtained after a 20 min centrifugation (20, 000 x g) was resuspended in 5 ml of diethylpyrocarbonate (DEPC)-treated water and the RNA was reprecipitated by incubation for 1 h at –20 ^{o}C after the addition of 300 µl of 4 M lithium chloride and 5 ml of cold absolute ethanol. After washing the pellet with 10 ml of cold 70% ethanol, the RNA was air dried at room temperature, resuspended in 1 ml of sterile distilled water, and stored at –80 ^{o}C until use.

Suppression Subtractive Hybridization (SSH) and Differentially Expressed Gene Cloning

For each cultivar, equal amounts of root RNA were pooled from the five sampled plants separately for each time point and SSH libraries were constructed by Evrogen (Moscow, Russia) in the following manner. Amplified ds cDNA was prepared from NemaTAM and Florunner RNA using the SMART approach (Zhu et al., 2001). SMART Oligo II oligonucleotide and cDNA synthesis (CDS) primer were used for first-strand cDNA synthesis. In both cases, first-strand cDNA synthesis involved 0.3 µg RNA in a total reaction volume of 10 µl. One microlitre of diluted (5x) first-strand cDNA was then used for PCR amplification with SMART PCR primer (Fig. 3-1). Nineteen PCR cycles (each cycle included 95 ^{o}C for 7 s; 65 ^{o}C for 20 s; 72 ^{o}C for 3 min) were performed. SMART-amplified cDNA samples of the tester (NemaTAM) and driver (Florunner) cDNA populations were digested with the restriction endonuclease *Rsa* I (Gibco) to obtain short, blunt-ended fragments (Fig. 3-1). Subtractive hybridization was performed using the SSH method in both directions (NemaTAM vs. Florunner and Florunner vs. NemaTAM) as described in Diatchenko et al. 1996 and 1999. Briefly, for each direction, two tester populations were created by ligation of different suppression adapters (Adapters 1 and 2R). These tester populations were mixed with 30X driver excess (driver cDNA had no adapters) in two separate tubes, denatured and allowed to renature. After the first hybridization, these two samples were mixed and hybridized together. Subtracted cDNA was then amplified by primary

and secondary PCR. The primary PCR consisted of 26 cycles with primer 1 (5'–CTAATACGACTCACTAT AGGGC-3') for subtracted NemaTAM cDNA and Florunner cDNA, respectively (Fig. 3-2). The secondary (nested) PCR consisted of 11 cycles with nested primers 1 (5'–TCGAGCGGCCGCCC GGGCAGGT–3') and 2R (5'–AGCGTGGTCGCGG CCGAGGT–3') for subtracted NemaTAM and Florunner cDNA samples, respectively (Fig. 3-2).

The Mirror Orientation Selection (MOS) procedure was performed for both SSH subtracted libraries as described in Rebrikov et al. (2000). Briefly, 22 PCR cycles with MOS PCR primer (5'–GGTCGCG GCCGAGGT–3') were performed for subtracted NemaTAM cDNA samples and 23 PCR cycles for subtracted Florunner cDNA samples. In order to obtain genes that were expressed in NemaTAM, and at lower levels or not at all in Florunner, the reverse subtraction was also carried out, using Florunner cDNAs as the tester. NemaTAM as tester and Florunner as driver and vice versa were used during forward and reverse subtractions. Two subtracted cDNA samples enriched with differentially expressed sequences (NemaTAM -specific and Florunner -specific) obtained by MOS PCR were used for library construction (Fig. 3-2). The PCR products after purification were cloned into the vector pAL17 (Evrogen) to produce the subtractive cDNA library, and subsequently transformed into *Escherichia coli*. In each case, approximately 40 ng of purified cDNA was cloned into pAL17 and used for *E. coli* transformation. For both libraries the ratio of white colonies (recombinant plasmids) to blue colonies (non-recombinant plasmids) was 70:30. Ninety-five percent of white colonies contained plasmids with insert.

Four hundred and eighty (five 96-well plates) randomly picked white colonies, each from both the tester (NemaTAM-specific) library and the driver (Florunner-specific) library, were used for differential screening which was performed by Evrogen (Moscow, Russia) as follows.

All plates were grown in 100 µl of LB-Amp (75 µg/ml) medium for six hours at 37 °C. Medium aliquots of 1 µl each were used for PCR amplification with pAL17 forward and pAL17 reverse primers. After the plates were supplied with 20% glycerol and stored at –70 °C, 2.0 µl of each PCR-amplified insert (about 100 ng DNA) was arrayed in a 96-well format onto duplicated nylon membranes (Hybond-N+ nylon membranes, Amersham International, Little Chalfont, Bucks, UK). After air drying, membranes were denatured in 0.6 M NaOH for 3 min, neutralized in 0.5 M Tris–HCl (pH 7.5) for 3 min, and rinsed in distilled water for 1 min. Samples were UV cross linked to membranes for 3 min at 70,000 micro-joules/cm^2 (SPECTROLINKER, XL-1000 UV Cross linker, Spectronics Corporation, Westbury, NY) and then stored at 4 ^oC for later use. Differential screening of potential clones that were highly specific to NemaTAM and Florunner was done by probing nylon membranes dotted by pin replication (Bio-Rad, Hercules, CA) of ordered clones of each 96-well micro-titration plate with ^{32}P-labeled (labeled by random-primed labeling using the Prime-a-Gene Labeling System; Promega, Madison, WI) subtracted cDNA of NemaTAM and Florunner. Based upon NemaTAM- and Florunner-specific signals, clones were selected and sequenced.

cDNA Sequence Analysis and Homology Search

The ESTs were sequenced using the M13 forward primer at the Interdisciplinary Center for Biotechnology Research (University of Florida, Gainesville, FL). Sequences were compared with sequences in the NCBI database using BLAST algorithms (http://www.ncbi.nlm.nih.gov/).

Northern Blot Analysis

Northern blot analysis was conducted by Evrogen (Moscow, Russia) as follows. Equal amounts of RNA (10 µg) from root samples collected at the five different time points (0, 12, 24, 48, and 72 h) and extracted as described above were separated on a 1.0% agarose/formaldehyde gel at 60 V for 2 h in 1X MOPS buffer followed by transfer to positively charged nylon

membranes (Hybond-NX, Amersham Biosciences, Piscataway, NJ) by upward capillary transfer with 20X SSC, and then UV cross linked for 3 min at 70,000 micro-joules/cm^2 (Spectrolinker, XL-1000 UV Cross linker, Spectronics Corporation, Westbury, NY). Specific probes labeled with *a*-^{32}P-dCTP were generated by purified PCR amplification from a plasmid containing the gene fragments of interest (NemaTAM: SSH-NE-PL5-A01, SSH-NE-PL5-C05, SSH-NE-PL5-E03, SSH-NE-PL5-F07; Florunner: SSH-FL-PL5-B06, SSH-FL-PL5-B10, SSH-FL-PL5-E06, SSH-FL-PL5-F01) of the relevant differentially expressed clones. After a 30-min pre-hybridization step performed in ExpressHyp solution (Clontech, Palo Alto, CA) and hybridization with the probe in the same solution for 1 h at 65 ^{o}C, the membrane was washed twice in 2X SSC and 0.1% SDS at room temperature for 15 min and then washed in 0.1X SSC and 0.1% SDS at 65 ^{o}C for 20 min.

RT-PCR

Reverse transcription of total RNA was carried out using iScript reverse transcriptase (Bio-Rad, Hercules, CA) with oligo-dT as the primer, according to the manufacturer's instructions. First-strand cDNA from 25 ng total RNA was used in each PCR reaction with the primer combinations listed in Table 3-1. PCR was carried out in a MJ-100 thermocycler (MJ Research, Watertown, MA) with the following program: 94 ^{o}C for 1min, followed by 25 or 30 cycles at 94 ^{o}C for 15 sec, 60 ^{o}C for 20 sec, 72 ^{o}C for 15 sec and a final extension at 72 ^{o}C for 1 min. PCR products were loaded onto a 1% agarose gel and run in 1X TBE containing ethidium bromide at 75 V for 3 hr.

Results

Differential Screening for cDNA Specific to NemaTAM and Florunner Following Inoculation with *Meloidogyne arenaria* race 1

In total, 960 clones from the NemaTAM- and Florunner- specific libraries were used for differential screening. Of these, 140 clones from the forward library showed a higher level of expression in the root-knot nematode resistant cultivar NemaTAM, while another 123 clones from the reverse library were more highly expressed in the root-knot nematode susceptible cultivar Florunner. A representative example highlighting the difference between the hybridization patterns with the forward and reverse probes is shown in Fig. 3-3. In order to reduce false positives to the lowest level, a series of differential screenings and identification were performed. Clones that showed different hybridization intensities between the two probes and which displayed at least a threefold higher transcription level between the cultivars were sequenced.

Sequence Analysis and Functional Annotation

A total of 263 sequences with an average insert size of 550 bp were obtained. These sequences were used in homology searches (Blastn and Blastx) of various databases (non-redundant sequences in NCBI, EST sequences in NCBI, and the potato and rice EST database at TIGR) with a cut off E-value of 1E-04 (Tables 3-2 and 3-3). A total of 132 sequences were identified using these criteria, 57 from the NemaTAM-specific library and 75 from Florunner-specific library. As shown in Table 3-2, ~ 30% of the NemaTAM-specific clones matched sequences found in *Arabidopsis thaliana* and another 21% could be found in other legumes such as *Medicago, Vigna, Pisum, Phaseolus* and *Glycine.* Only one of the NemaTAM-specific sequences belonged to *Arachis hypogaea* (~ 2%). Approximately 32% of the clones have sequences with significant homology to genes found in other plants such as *Zea diploperennis*,

Bowringia mildbraedii, Sorghum bicolor, Solanum cardiophyllum, Zingiber officianalis, Nicotiana tabacum, Prunus persica, Cucurbita pepo and *Trifolium pratense*. The remaining clones, 15% have sequences related to genes found in other organisms such as *Porcupine* (rodent), *Drosophila pseudoobscura* (fruit fly), *Burkholderia ambifaria* (bacterium), and *Cholorobium phaebacteroides* (bacterium).

As illustrated in Table 3-3, ~ 23% of the Florunner-specific sequences were homologous to *Arabidopsis thaliana* and another 40% belonged to other legumes such as *Medicago truncatula, Vigna radiata, Pisum sativa, Phaseolus vulgaris,* and *Glycine max.* Only 3% of the Florunner-specific sequences belonged to *Arachis hypogaea*. Twenty-seven percent of the other clones have sequences with significant homology to genes found in *Sophora alopecuroides*, *Pterocarpus rotundifolius, Cucurbita pepo, Oryza sativa, Nicotiana tabacum, Solanum cardiophyllum, Prunus persica, Beta procumbens, Vitis aestivalis, Gymnodenia conopsea,* and *Malus domestica*. The remaining clones, 3% have sequences related to genes found in other organisms such as *Strongylocentrotus purpuratus* (sea urchin).

Putative functions of ~ 55% (49 out of 90) of the NemaTAM-specific sequences and 78% (65 out of 83) of the Florunner-specific sequences were assigned (Tables 3-2 and 3-3). The NemaTAM- and Florunner-specific sequences were classified into six primary functional categories according to the putative function of their homologous genes in the databases, and the distribution of these genes is illustrated in Fig. 3-4. For NemaTAM, the largest set of genes (38%) was assigned to the response to stress/plant defense category, followed by genes with unknown function (45%), metabolism (9%), signal transduction and cellular communication (4%), transcriptional activity (2%), and proteolysis (2%). For Florunner, the largest set of genes (30%) was also assigned to the response to stress/plant defense category, followed by genes

involved in signal transduction and cellular communication (24%), unknown function (22%), transcriptional activity (11%), hydrolase activity (7%), and metabolism (6%). The sequences of both NemaTAM and Florunner that showed no significant homology to sequences deposited in public databases are listed in APPENDIX A and B, respectively. Looking more closely at the major functional category, response to stress/plant defense, it can be broken down into the distribution illustrated in Fig. 3-5. For NemaTAM, pathogenesis related (PR) transcripts occupied the largest percentage of the five categories (36%). This is followed by genes involved in oxidative stress (23%); universal stress (23%), lectins (9%), and senescence associated function (9%). For Florunner, lectins occupied the largest percentage (52%), followed by root-knot nematode response activity (24%), oxidative stress (20%), and senescence associated function (4%).

Validation of Differential Gene Expression Results

Northern blot analysis was performed to validate the differential screening results. Four clones from each subtracted library were used for analysis, and the results are shown in Figs. 3-6 and 3-7. Clones selected from the NemaTAM-subtracted library (SSH-NE-PL5-A01, SSH-NE-PL5-C05, SSH-NE-PL5-E03, SSH-NE-PL5-F07; Fig. 3-6) and the Florunner subtracted library (SSH-FL-PL5-B06, SSH-FL-PL5-B10, SSH-FL-PL5-E06, SSH-FL-PL5-F01; Fig. 3-7) showed appropriate levels of differential expression. NemaTAM-specific clones when blasted in GenBank showed homology to genes encoding proteins of unknown function. Among Florunner-specific clones: SSH-FL-PL5-B06 showed homology to a catalase encoding gene, SSH-FL-PL5-B10 to a gene encoding an unknown protein and the remaining two clones, SSH-FL-PL5-E06, SSH-FL-PL5-F01, showed homology to genes encoding the Hs1Pro1 protein and a syringolide-induced protein, respectively.

Further validation of randomly selected differential clones was done by RT-PCR. Total RNA from NemaTAM and Florunner was used. As a control probe, the highly conserved ribosomal subunit 18S, which is expressed in equal amounts in both cultivars, was used (Figs. 3-6 and 3-7). Once again, these clones exhibited cultivar-specific expression as originally determined by SSH.

Discussion

The transcription profiles of infected roots of the root-knot nematode resistant peanut cultivar NemaTAM and the susceptible cultivar Florunner were compared by SSH analysis. The number of transcripts coding for proteins involved in plant defense (including PR proteins) was comparatively higher in NemaTAM than in Florunner. These transcripts include the pathogenesis related mRNAs encoding a major timothy grass pollen allergen, pathogenesis-related protein 1, S-adenosyl-L-methionine: trans-caffeoyl-CoenzymeA3-O-methyltransferase, disease resistance protein (LRR), arachidic acid-induced DEA1 protein, and an ATPRB1/Putative pathogenesis-related protein. In addition, substantially more clones containing cDNAs encoding enzymes responsible for mitigating oxidative stress such as oxidoreductase/zinc ion, NADH: ubiquinone oxidoreductase, copper chaperone homolog CCH/Detoxifies heavy metals, NADPH peroxidase/oxidase, and patatin-like protein were more highly expressed in NemaTAM than in Florunner.

The expression of PR proteins in the roots of NemaTAM, and possibly other parts of the plant, is likely responsible for restricting the entry, establishment and development of second-stage juveniles of *M. arenaria* race 1 (Bendezu and Starr, 2003). They may also be responsible for the failure of the root-knot nematode to complete its life cycle in the giant cells. In plants, formation of PR proteins plays an important role in both compatible and incompatible interactions and the defense against pathogens. This response to pathogen infection is usually

systemic because the PR proteins not only accumulate at sites of pathogen localization, but also in non-inoculated parts of the plant (Zinoveva et al., 2004). Additionally, Zinoveva et al. (2001) observed that PR-related enzyme activity increases upon nematode infection, and that it is higher in nematode resistant plants compared to their susceptible counterparts under the same conditions. Similarly, an earlier study by Qui et al. (1997) on two soybean cultivars which differ in their susceptibility to *M. incognita* infection revealed that higher PR protein activity conferred resistance to root-knot nematodes. These results indicate that PR proteins are involved in defense against plant-parasitic nematodes and probably play a major role in NemaTAM's resistance to the root-knot nematode.

It has also been well documented that an oxidative burst plays a central role in plant defense against pathogens, and that reactive oxygen species cause damage to the pathogen (Apel and Hirt, 2004). Therefore, it is not surprising that NemaTAM highly expresses a number of clones encoding peroxidases, oxidoreductases, and heavy metal detoxifiers (Table 3-2). Moreover, Sabine et al. (1997) using pathogenesis-related experiments suggested the involvement of oxidoreductases in defense against pathogens.

NemaTAM also expressed a number of Universal Stress Proteins (USP); however USP expression was absent in Florunner. In prokaryotes, USP genes are known to be stimulated and required for stress resistance, survival during growth limitation, and oxidative stress resistance (Nystrom and Neidhart, 1994; Nachin et al., 2005).

Taken together, differential expression of PR and oxidative stress responsive proteins in NemaTAM suggests that these proteins play a prominent role in defense against *M. arenaria* race 1. Because a higher level of expression of PR proteins requires increased levels of salicylic acid (SA), which is usually followed by universal and oxidative stress responses, it is proposed

that SA receives a signal from R-genes and triggers universal stress and oxidative stress responsive genes further triggering downstream expression of pathogenesis-related genes. Therefore, the SA pathway might be operating in NemaTAM for resistance against *M. arenaria*. This hypothesis needs to be tested by studying the levels of specific PR gene expression both in uninoculated and root-knot nematode inoculated NemaTAM and Florunner.

In addition to the genes described above, several other cDNAs were identified that are potentially related to a root-knot nematode defense response. For example, two clones SSHPL1E05 and SSHPL1D11 (Table 3-2), which encode a CCHC-type cytokinin binding protein CBP57 and a transmethyl regulation protein, respectively may interact with other defense genes triggering downstream gene expression. Three putative signaling proteins (SSHPL1D01, SSHPL1B11 and SSHPL1B06 (Table 3-2) may also be involved in cellular communication or signal transduction in plant defense.

Among the genes that were represented more often in Florunner than in NemaTAM, it is most striking that there are no PR transcripts encoding plant defense proteins. However, non-PR transcripts, such as those for lectins and Hs1pro1, were highly expressed in Florunner. There are several reports of lectins having biological activity against nematodes. For example, Marban-Mendoza et al. (1987) reported that concanavalin A suppresses *M. incognita* when used as a soil amendment in tomato. Burrows et al. (1998) reported that snowdrop lectin (GNA) when expressed in potato decreased the number of females of *Globodera pallida*. Lectins are believed to have potential as external anti-nematode effectors by causing behavioral disruption (Atkinson et al., 2003). Although the predicted protein to be encoded by *Hs1pro-1* has a leucine-rich region and a putative transmembrane domain, it does not belong to the NBS-LRR class of plant *R* genes nor does it fit any other existing R protein class (Thurau et al., 2003). Therefore, it has been

proposed that *Hs1pro-1* represents an entirely new class of *R* genes (Jung et al., 1998). Thurau et al. (2003) also reported that the promoter of *Hs1pro-1* upon activation triggered nematode-responsive gene expression in sugar beet (*Beta vulgaris* L.) and *Arabidopsis thaliana*. That study clearly demonstrated that upon nematode infection, *Hs1pro-1* was locally induced; and its transcriptional activity reached its maximum one day after nematode infection. Based on these results, it appears that enhanced expression of *Hs1pro-1* in feeding structures might be crucial for nematode resistance. Taking these results into consideration Florunner may exhibit resistance to other plant-parasitic nematodes such as *Globodera pallida*, but remain susceptible to *M. arenaria* due to the absence of specific *R* genes.

Florunner expressed a number of genes involved in signal transduction and cellular communication, such as the calcium-binding EF-hand and syringolide-induced (SIH) proteins. Analysis by Day et al. (2002) indicated that EF-hand-containing proteins are probably the key transducers mediating Ca^{2+} action. Hagihara et al. (2004) reported that Ca^{2+} is required for hypersensitive cell death (HCD) and the induction of SIH genes. These results suggest that the induction of SIH genes could be activated through the syringolide specific signaling pathway and the SIH gene products may play an important role in the processes of HCD. Based on this study, it is proposed that upon the perception of stress from primary and secondary signaling molecules following root-knot nematode infection in Florunner there is an increase in calcium concentration activating EF-hand-containing proteins that are probably the key transducers mediating Ca^{2+} action. This event is followed by syringolide-specific signaling pathway and the SIH gene products may play an important role in the processes of HCD.

Among additional Florunner-specific transcripts that amplified were *Phi* and *XET* genes reported to be regulated by ABA (Sano and Nagata, 2002; Wu et al., 1994). It appears that ABA

induced gene expression of the *Phi-1* gene alleviate fluctuations in intracellular pH (Sano and Nagata, 2002). These results suggest that Florunner may counteract the non-synchronous cell division caused by root-knot nematode attack by expression of *Phi-1*. It can be further proposed from the above studies that stress due to root-knot nematode attack might result in ABA-mediated acidification of the cytoplasm. Cytoplasmic acidification further triggers *Phi-1* and *Phi-2* genes which are reported to induce semi-synchronous cell division resulting in giant cells/feeding sites for *M. arenaria* (Sano and Nagata, 2002). Further functional characterization of the *Phi-1* gene should unravel the role of phosphate in inducing cell division in plant cells.

Xyloglucan endotransglycosylase (XET) has been proposed to cause cell wall-loosening by cleaving xyloglucan molecules and there by promoting micro fibril separation (Smith and Fry, 1989; Nishitani and Tominaga, 1991; Fry et al., 1992). On the other hand, Sharp et al. (1994) reported that exogenous application of ABA restored the activity of XET which in turn resulted in recovery of the root elongation rate. Based on these previous reports and expression analysis of XET, It is proposed that XET gene expression may be regulated via ABA signaling by one of the transcription factors of *M. arenaria* to loosen the cell walls for efficient feeding of cell contents.

In summary, 263 differentially expressed genes were detected in two near-isogenic peanut cultivars, NemaTAM and Florunner, which differ only with respect to their resistance to *M. arenaria* race 1. The major conclusion of this study is that, in addition to the expression of a number of well-characterized PR proteins, high-level expression of genes encoding USPs may be major factors in rendering NemaTAM resistant to infection by the root-knot nematode *M. arenaria* race 1. These transcripts were absent in the root-knot nematode susceptible cultivar

Florunner. However, the expression of lectins, *Hs1pro-1* and SIH responsive genes in Florunner indicates a basal level of plant defense against *M. arenaria*.

Elucidating the molecular basis of root-knot nematode resistance found in NemaTAM should contribute to the understanding of nematode-plant interactions and assist in breeding future nematode-resistant peanut cultivars. Furthermore, this information provides candidate genes that can be further analyzed for their utility against root-knot nematodes.

Table 3-1. RT-PCR primer combinations.

Clone	Forward Primer	Reverse Primer
SSH[1]-NE[2]-PL5[3]-A01[4]	5'-TACTCTGGCATCGCAAAATG-3'	5'-AGGAGGTTCTGCTCCAATGA-3'
SSH-NE-PL5-C05	5'- GCCGAGGTACACATCAGTGG -3'	5'- GGCCGAGGTACATAAGGAGA -3'
SSH-NE-PL5-E03	5'- GTATGGCAAAGCCTTCCTGA -3'	5'- CGAAATAGGGGAACACATGG -3'
SSH-NE-PL5-F07	5'- CGCGGGGATATACAATCTCA -3'	5'- CACAATTGGACGTGGATACG -3'
SSH-FL[5]-PL5-B06	5'- ACCAACTTGCAACACAACCA -3'	5'- TTGCAGAGAGGTTTGTGGAAT -3'
SSH-FL-PL5-B10	5'- GGCAATTCTTTCCGTTTTCA -3'	5'- GTCATCAGGGCCGTTAGAGA -3'
SSH-FL-PL5-E06	5'- CCAGATTGTGGAGTCCTGGT-3'	5'- CACCTCCACTAGCTCCTTCG -3'
SSH-FL-PL5-F01	5'-TGCAAATGGTGCTTCTCTTG -3'	5'-AACCAAGCCTTCAGCAACAC-3'

[1]SSH = Suppression Subtractive Hybridization; [2]NE = NemaTAM; [3]PL=Plate 5; [4]AO1= Clone A01 in plate 5; [5]FL=Florunner.

Table 3-2. Identification of fifty-seven EST clones from the root-knot nematode resistant cultivar NemaTAM.

Clone ID	Accession # of EST clone	Accession # of matching sequence	E-value	Source of matching sequence	Function
			Response to Stress		
Pathogenesis related (PR) proteins					
SSHPL1H08	Ne469375	INIOB	3E-04	*Phleum pretense*	Timothy grass pollen allergen
SSHPL1H03	Ne469380	ABA34077	8E-06	*Zea diploperennis*	Pathogenesis-related protein 1
SSHPL1G07	Ne469388	AAA62426	4E-05	*Arabidopsis thaliana*	S-adenosyl-L-methionine:trans-caffeoyl-CoenzymeA3-O-methyltransferase
SSHPL1E07	Ne469412	XP_476483	1E-15	*Oryza sativa*	Putative Pathogenesis-related protein
SSHPL1D09	Ne469422	CAA65419	1E-17	*Arabidopsis thaliana*	Pathogenesis-related protein
SSHPL1C07	Ne469436	ABD28507	4E-11	*Medicago truncatula*	Disease Resistance Protein(LRR)
SSHPL1B10	Ne469445	CAI51313	4E-22	*Capsicum chinense*	Arachidic acid-induced DEA1 protein
SSHPL2A07	Ne472920	XP_476500	1E-15	*Oryza sativa*	Pathogenesis-related protein 1
SSHPL2A04	Ne472923	NP_179064	8E-08	*Arabidopsis thaliana*	ATPRB1/Putative pathogenesis-related protein
SSHPL2A01	Ne472926	CAI51313	2E-14	*Capsicum chinense*	Arachidic acid-induced DEA1 protein
SSHPL2B10	Ne472984	AA033592	5E-08	*Arachis hypogaea*	Putative cold stress responsive protein
SSHPL2C01	Ne472981	AAW38998	8E-15	*Arabidopsis thaliana*	Pathogenesis related protein
Lectins					
SSHPL1H11	Ne469372	1QMOH	4E-07	*Dolichos lab lab*	Lectin
SSHD07	Ne469424	P42088	7E-35	*Bowringia mildbraedii*	Lectin
SSHPL2A03	Ne472924	P42088	1E-40	*Bowringia mildbraedii*	Lectin
Senescence associated function					
SSHPL2B03	Ne472991	AAR13310	4E-35	*Phaseolus vulgaris*	B12D-Like protein
SSHPL2B01	Ne472993	AAR13310	3E-35	*Phaseolus vulgaris*	B12D-Like protein
SSHPL2D01	Ne472969	AAR13310	8E-35	*Phaseolus vulgaris*	B12D-Like protein

Table 3-2. (Continued)

Clone ID	Accession # of EST clone	Accession # of matching sequence	E-value	Source of matching sequence	Function
Oxidative stress					
SSHPL1A07	Ne469384	NP_197201	4E-30	*Arabidopsis thaliana*	Oxidoreductase/Zinc ion
SSHPL1F10	Ne469397	EAL33043	2E-06	*Drosophila pseudoobscura*	NADH:Ubiquinone oxidoreductase
SSHPL1F09	Ne469398	1OXWC	7E-15	*Solanum cardiophyllum*	Patatin-like Protein1
SSHPL1E01	Ne469418	CAA89262	8E-87	*Arabidopsis thaliana*	NADH Oxidoreductase
SSHPL1C11	Ne469432	AAF15286	3E-35	*Glycine max*	Copper Chaperone Homolog CCH/Detoxifies heavy metals
SSHPL1C02	Ne469441	NP910315	1E-05	*Oryza sativa*	NADPH peroxidase/Oxidase
SSHPL1B12	Ne469443	NP_199172	2E-14	*Sorghum bicolor*	Patatin-like Protein
SSHPL2C03	Ne472979	AAF15286	7E-38	*Glycine max*	Copper Chaperone Homolog CCH/Detoxifies heavy metals
Universal stress					
SSHPL1A02	Ne469369	NP_566108	1E-31	*Arabidopsis thaliana*	Universal stress protein (Usp)
SSHPL1G08	Ne469387	ABA34077	8E-06	*Cholorobium phaebacteroides*	Universal stress protein (Usp)
SSHPL1C12	Ne469431	ABE85050	8E-29	*Medicago truncatula*	Universal stress protein (Usp)
SSHPL1C08	Ne469435	ABE85050	3E-29	*Medicago truncatula*	Universal stress protein (Usp)
SSHPL1C04	Ne469439	ABE85050	2E-28	*Medicago truncatula*	Universal stress protein (Usp)
SSHPL1C03	Ne469440	ABE85050	4E-29	*Medicago truncatula*	Universal stress protein (Usp)
SSHPL1BO7	Ne469448	NP_566108	2E-29	*Arabidopsis thaliana*	Universal stress protein (Usp)
SSHPL1BO7	Ne469448	NP_566108	1E-29	*Arabidopsis thaliana*	Universal stress protein (Usp)

Table 3-2. (Continued)

Clone ID	Accession # of EST clone	Accession # of matching sequence	E-value	Source of matching sequence	Function
			Transcriptional Activity		
SSHPL1E05	Ne469414	ABE83455	7E-07	*Medicago truncatula*	Zinc finger, CCHC-type
SSHPL1D11	Ne469420	BBA03710	3E-35	*Nicotiana sylvestris*	Cytokinin binding proteinCBP57
			Signal Transduction and Cellular Communication		
SSHPL1D01	Ne469430	NP_178306	1E-68	*Arabidosis thaliana*	Transporter protein/Putative endomembrane protein
SSHPL1B11	Ne469444	XP_470213	5E-46	*Oryza sativa*	Tonoplast intrinsic protein
SSHPL1B06	Ne469449	BAD22802	8E-59	*Prunus persica*	Nitrate transporter
			Metabolism		
SSHPL1B02	Ne469357	NP_200261	3E-78	*Arabidopsis thaliana*	Flavodoxin-like Quinone reductase 1
SSHPL1H07	Ne469376	4MDHB	2E-11	*Sus scrofa* heart (procupine)	Cytoplasmic Malate dehydrogenase
SSHPL1G11	Ne469384	ZP_00688862	1E-58	*Burkholderia ambifaria*	5-methyl tetrahydropteroyltriglutamate homocysteine
SSHPL1F08	Ne469399	ZP_01043980	1E-20	*Idiomarina baltica*	Carbamoylphosphate synthase small subunit
SSHPL1E04	Ne469415	NP_199172	5E-16	*Arabidopsis thaliana*	nutrient reservoir/patatin like phospholipase
SSHPL1E03	Ne469416	BAD38550	7E-49	*Oryza sativa*	nutrient reservoir/putative patatin like homolog
SSHPL1B04	Ne469451	AT46998	3E-20	*Glycine max*	Triosephosphate isomerase
SSHPL2C02	Ne472980	AAY86360	1E-38	*Acacia* species	Cinnamoyl-CoA reductase

Table 3-2. (Continued)

Clone ID	Accession # of EST clone	Accession # of matching sequence	E-value	Source of matching sequence	Function
				Proteolysis	
SSHPL2B12	Ne472982	NP_565330	2E-57	*Arabidopsis thaliana*	Peptidase/Subtilase/proteolysis
SSHPL2D06	Ne472964	BAE71300	3E-23	*Trifolium pretense*	Putative snRNP 70K protein
				Unknown Function	
SSHPL1H06	Ne469377	1OXWC	2E-07	*Solanum cardiophyllum*	Hypothetical protein
SSHPL1B01	Ne469358	NP_189632	3E-28	*Arabidopsis thaliana*	Unknown protein
SSHPL1A04	Ne469367	NP_198805	4E-45	*Arabidopsis thaliana*	Unknown protein
SSHPL1A03	Ne469368	NP_568343	8E-06	*Arabidopsis thaliana*	Unknown protein
SSHPL1G06	Ne469389	EAL31846	2E-49	*Drosophila pseudoobscura*	GA14712-PA gene product
SSHPL1D05	Ne469426	NP_910315	1E-05	*Oryza sativa*	Unknown protein
SSHPL2C04	Ne472978	NP_568343	1E-05	*Arabidopsis thaliana*	Unknown protein
SSHPL2D08	Ne472962	NP_910315	6E-06	*Oryza sativa*	Unknown protein

Table 3-3. Identification of seventy-five EST clones from the root-knot nematode susceptible cultivar Florunner.

Clone ID.	Accession # of EST clone	Accession # of matching sequence	E-value	Source of matching sequence	Function
			Response to Stress		
Lectins					
SSHPL3B02	FL468983	AAY68291	2E-06	*Sophora alopecuroides*	Lectin
SSHPL3A05	FL468992	AAY68291	2E-29	*Sophora alopecuroides*	Lectin
SSHPL3H07	FL469002	AAY68291	1E-04	*Sophora alopecuroides*	Lectin
SSHPL3H03	FL469006	ABE89758	2E-18	*Medicago truncatula*	Concanavalin A-like lectin / glucanase
SSHPL3G05	FL469016	AAT57665	3E-45	*Pterocarpus rotundifolius*	Lectin
SSHPL3D12	FL469045	AAT57665	1E-45	*Pterocarpus rotundifolius*	Lectin
SSHPL3D11	FL469046	AAT57665	2E-42	*Pterocarpus rotundifolius*	Lectin
SSHPL3C12	FL469057	AAT57665	5E-43	*Pterocarpus rotundifolius*	Lectin
SSHPL3C10	FL469059	AAT57665	4E-44	*Pterocarpus rotundifolius*	Lectin
SSHPL3C05	FL469064	AAT57665	2E-45	*Pterocarpus rotundifolius*	Lectin
SSHPL3C02	FL469067	AAT57665	3E-47	*Pterocarpus rotundifolius*	Lectin
SSHPL2F07	FL472939	AAT57665	5E-46	*Pterocarpus rotundifolius*	Lectin
SSHPL2E12	FL472946	AAT57665	1E-47	*Pterocarpus rotundifolius*	Lectin
Nematode responsive gene activity					
SSHPL3E02	FL469043	AAG44839	6E-25	*Glycine max*	Putative Hs1pro1-like receptor
SSHPL3D07	FL469050	AAG44839	3E-23	*Glycine max*	Putative Hs1pro1-like receptor
SSHPL3D05	FL469052	AAG44839	1E-26	*Glycine max*	Putative Hs1pro1-like receptor
SSHPL3C09	FL469060	AAF67003	4E-07	*Pisum sativa*	Putative Hs1pro1-like homolog
SSHPL2E08	FL472950	AAG44839	8E-27	*Glycine max*	Putative Hs1pro1-like receptor
SSHPL3A10	FL468987	AAZ39087	3E-25	*Beta procumbens*	Nematode resistance protein
Senescence associated function					
SSHPL3F12	FL469021	BAD42919	2E-52	*Arabidopsis thaliana*	Senescence-associated protein

Table 3-3 (Continued)

Clone ID.	Accession # of EST clone	Accession # of matching sequence	E-value	Source of matching sequence	Function
Oxidative stress					
SSHPL3D04	FL469053	P48350	4E-51	*Cucurbita pepo*	Catalase isozyme1
SSHPL3A12	FL468985	AAZ29552	5E-76	*Oryza sativa*	Catalase-C
SSHPL3H11	FL468998	ABE83062	8E-11	*Medicago truncatula*	Heavy metal detoxification protein
SSHPL2F04	FL472942	AAC06243	3E-08	*Nicotiana tabacum*	Osmotic stress-induced protein
SSHPL2E11	FL472947	CAD42909	9E-69	*Prunus persica*	Catalase
Transcriptional Activity					
SSHPL3A02	FL468995	ABE86987	6E-13	*Medicago truncatula*	DNA-binding WRKY
SSHPL3H09	FL468900	ABE86850	5E-12	*Medicago truncatula*	Basic helix loop helix (bHLH) factor
SSHPL3H06	FL469003	ABE_93963	9E-47	*Medicago truncatula*	Zinc finger,C2H2-type
SSHPL3H05	FL469004	ABE86271	2E-33	*Medicago truncatula*	Homeodomain related
SSHPL3F04	FL469029	ABD66513	1E-30	*Gymnodenia conopsea*	Eukaryotic translation initiation factor
SSHPL3E05	FL469040	ABE85589	2E-05	*Medicago truncatula*	Zinc finger protein
SSHPL3D03	FL469054	ABE93963	3E-50	*Medicago truncatula*	Zinc finger,C2H2-type
SSHPL3C11	FL469058	AAR92477	1E-18	*Vitis aestivalis*	Putative WRKY transcription factor 30
SSHPL3B12	FL469069	AAM15234	8E-06	*Arabidopsis thaliana*	Putative bHLH transcription factor

Table 3-3 (Continued)

Clone ID.	Accession # of EST clone	Accession # of matching sequence	E-value	Source of matching sequence	Function
Signal Transduction and Cellular Communication					
Calcium-binding EF-Hand					
SSHPL3B01	FL468985	ABE78935	3E-46	*Medicago truncatula*	Calcium-binding EF-hand
SSHPL3A11	FL468986	ABE78935	1E-45	*Medicago truncatula*	Calcium-binding EF-hand
SSHPL3G08	FL469013	ABE78935	6E-39	*Medicago truncatula*	Calcium-binding EF-hand
SSHPL3G07	FL469014	NP_173355	8E-26	*Arabidopsis thaliana*	DNA binding protein
SSHPL3F09	FL469024	ABE78935	1E-29	*Medicago truncatula*	Calcium-binding EF-hand
SSHPL3E03	FL469042	ABE78935	1E-32	*Medicago truncatula*	Calcium-binding EF-hand
SSHPL3C06	FL469063	ABE78935	3E-30	*Medicago truncatula*	Calcium-binding EF-hand
SSHPL2F03	FL472943	ABE78935	2E-32	*Medicago truncatula*	Calcium-binding EF-hand
SSHPL2D10	FL472960	ABE78935	5E-37	*Medicago truncatula*	Calcium-binding EF-hand
Phi-1 protein (Phosphate induced activity)					
SSHPL3A07	FL468990	CAJ13706	1E-46	*Capsicum chinense*	Phi-1 protein
SSHPL3A01	FL468996	CAJ13706	2E-16	*Capsicum chinense*	Phi-1 protein
SSHPL3H01	FL469008	CAJ13706	7E-115	*Capsicum chinense*	Phi-1 protein
SSHPL3G09	FL469012	CAJ13706	3E-30	*Capsicum chinense*	Phi-1 protein
SSHPL2F08	FL472938	BAA33810	2E-28	*Nicotina tabacum*	Phi-1 protein
Auxin related activity					
SSHPL3G05	FL469018	O24543	5E-43	*Vigna radiate*	Auxin induced protein 22E
SSHPL3D06	FL469051	AAZ20292	2E-11	*Arachis hypogaea*	Auxin repressed protein
SSHPL3C08	FL469061	AAZ20292	2E-25	*Arachis hypogaea*	Auxin repressed protein
Syringolide-induced proteins					
SSHPL3G02	FL469019	BAB86896	2E-55	*Glycine max*	Syringolide-induced protein 13-1-1
SSHPL3F10	FL469023	BAB86896	4E-38	*Glycine max*	Syringolide-induced protein 13-1-1
SSHPL2E10	FL472948	BAB86896	6E-59	*Glycine max*	Syringolide-induced protein 13-1-1

Table 3-3 (Continued)

Clone ID.	Accession # of EST clone	Accession # of matching sequence	E-value	Source of matching sequence	Function
Nodulin					
SSHPL3F07	FL469026	AAC32828	9E-05	*Glycine max*	Nodulin
				Hydrolase Activity	
SSHPL3F05	FL469028	NP_172939	8E-43	*Arabidopsis thaliana*	Hydrolase
SSHPL3DO9	FL469049	NP_173266	1E-53	*Arabidopsis thaliana*	Hydrolase
SSHPLED01	FL469056	NP_173266	3E-52	*Arabidopsis thaliana*	Hydrolase
SSHPL3C01	FL469068	NP_173266	1E-42	*Arabidopsis thaliana*	Hydrolase
SSHPL2F02	FL472944	NP_173266	1E-51	*Arabidopsis thaliana*	Hydrolase
SSHPL2D11	FL472960	NP_173266	5E-50	*Arabidopsis thaliana*	Hydrolase
				Unknown Function	
SSHPL3H10	FL468999	XP_792559	1E-09	*Strongylocentrotus purpuratus*	Hypothetical protein
SSHPL3F01	FL469032	AAU44522	4E-06	*Arabidopsis thaliana*	Hypothetical protein AT4G27810
SSHPL3E12	FL469033	ABE92227	3E-46	*Medicago truncatula*	Conserved hypothetical protein
SSHPL3B03	FL469078	ABE92227	4E-34	*Medicago truncatula*	Conserved hypothetical protein
SSHPL3G06	FL469015	NP_564307	2E-63	*Arabidopsis thaliana*	Unknown protein
SSHPL3G06	FL469044	NP_566063	3E-44	*Arabidopsis thaliana*	Unknown protein
SSHPL3B08	FL469073	NP_196762	1E-39	*Arabidopsis thaliana*	Unknown protein
SSHPL2F09	FL472937	NP_179949	4E-16	*Arabidopsis thaliana*	Unknown protein
SSHPL2E05	FL472953	NP_182061	3E-17	*Arabidopsis thaliana*	Unknown protein
SSHPL2E04	FL472954	NP_195718	1E-05	*Arabidopsis thaliana*	Unknown protein

Table 3-3 (Continued)

Clone ID.	Accession # of EST clone	Accession # of matching sequence	E-value	Source of matching sequence	Function
			Others		
SSHPL3D02	FL469055	AAN07898	1E-100	*Malus domestica*	Xyloglucan endotransglycosylase (XET)
SSHPL2F10	FL472936	ABC68415	2E-39	*Glycine max*	Cytochrome P450 Monoxygenase
SSHPL2D09	FL472961	ABE82347	4E-21	*Medicago truncatula*	Las1-like protein/ Cell morphogenesis and cell surface growth
SSHPL2E06	FL472952	NP_181933	1E-10	*Arabidopsis thaliana*	Riboflavin synthase

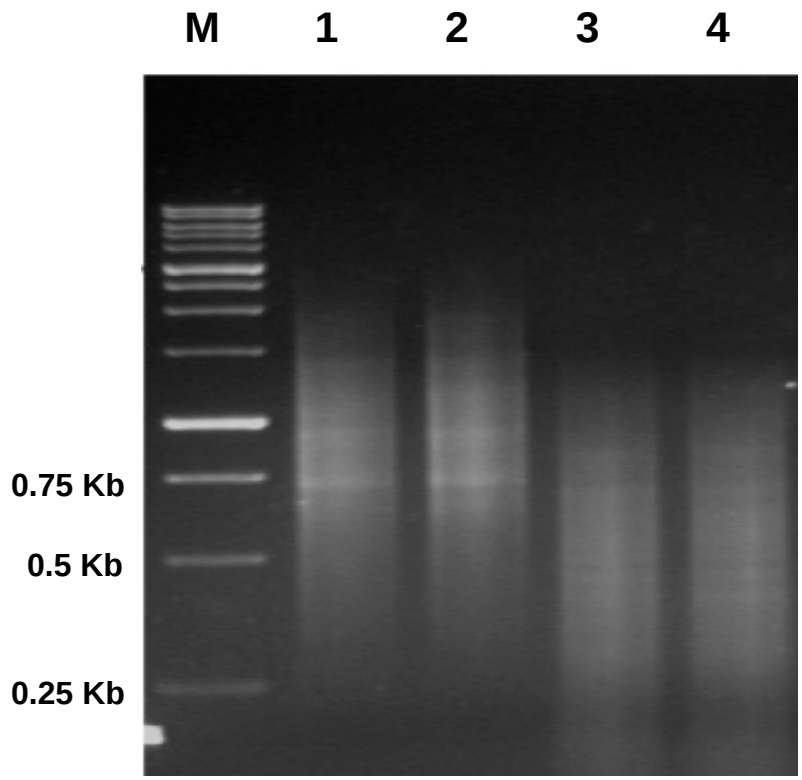

Figure 3-1. Double-stranded cDNA synthesis and Rsa I digestion. Lanes: M = 1 Kb DNA size marker; 1 = SMART-amplified NemaTAM cDNA (tester); 2 = SMART-amplified Florunner cDNA (driver); 3 = Rsa I digested NemaTAM cDNA; 4 = Rsa I digested Florunner cDNA.

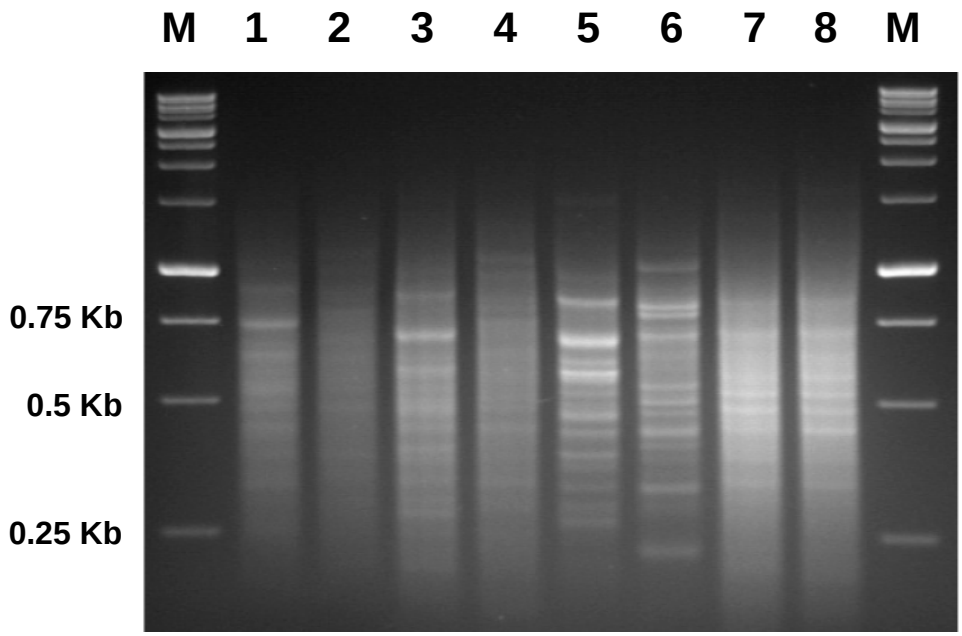

Figure 3-2. Agarose gel electrophoresis of primary and secondary PCR products. Lanes: M = 1 Kb DNA size markers; 1 = Primary PCR of NemaTAM subtracted cDNA; 2 = Primary PCR of Florunner subtracted cDNA; 3 = Secondary PCR of NemaTAM subtracted cDNA; 4 = Secondary PCR of Florunner subtracted cDNA; 5 = Mirror Orientation Selection PCR of NemaTAM subtracted cDNA; 6 = Mirror Orientation Selection PCR of Florunner subtracted cDNA; 7 = Unsubtracted NemaTAM cDNA; 8 = Unsubtracted Florunner cDNA.

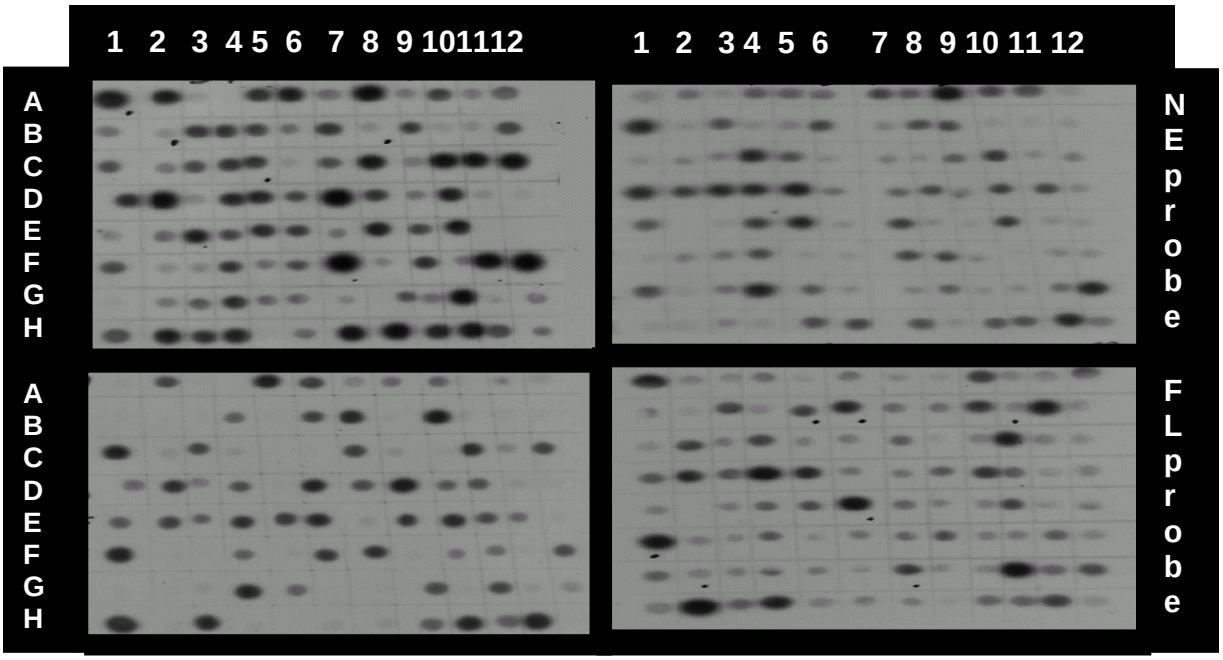

Figure 3-3. Differential screening results. Ninety-six PCR-amplified inserts each of NemaTAM and Florunner (about 100 ng DNA) were arrayed in a 96-well format onto duplicated nylon membranes and hybridized with either P-32-labelled subtracted cDNAs of NemaTAM (NE probe) or Florunner (FL probe).

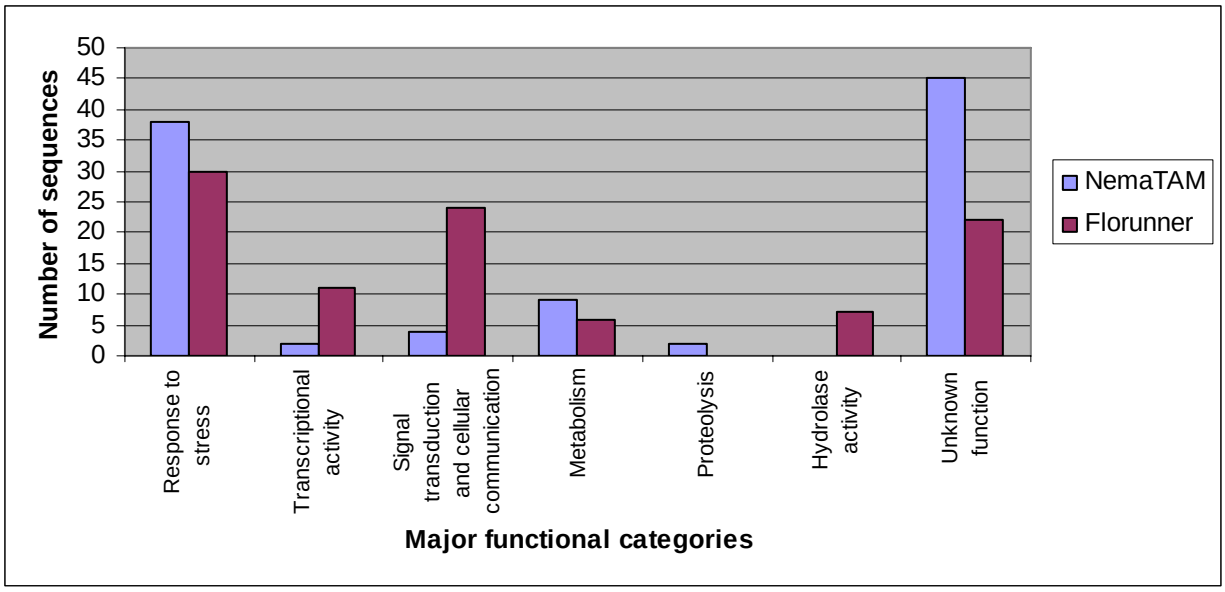

Figure 3-4. Comparison of major functional categories of NemaTAM and Florunner expressed sequences. Note: The unknown function category is comprised of clones which showed significant homology to hypothetical and unknown proteins, and the clones which gave no significant hits in GenBank.

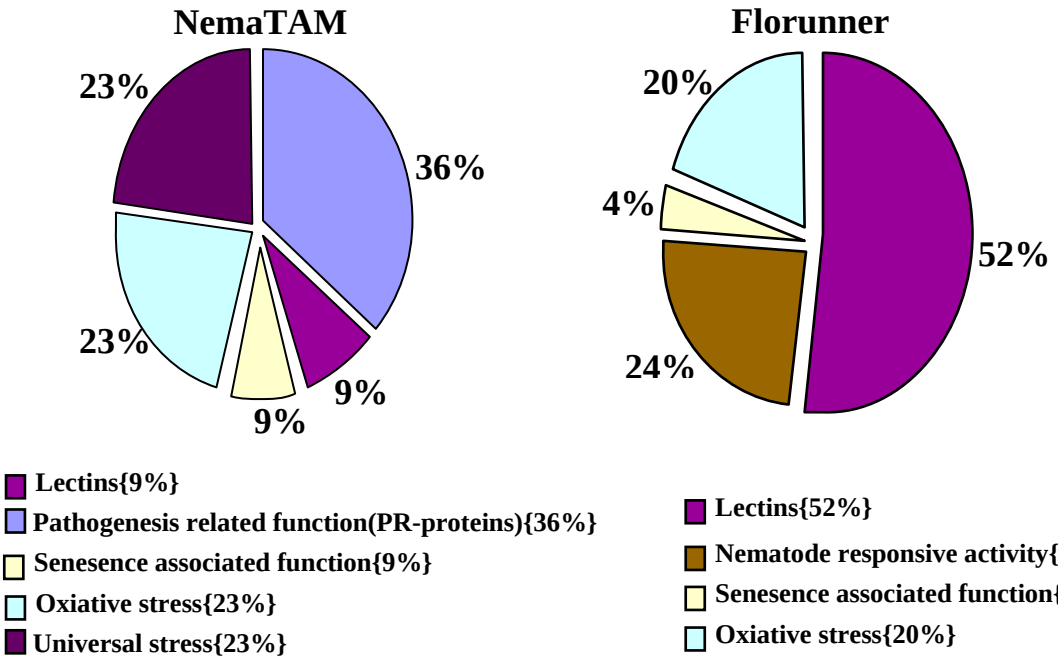

Figure 3-5. Subcategories within the response to stress plant defense functional category for NemaTAM and Florunner.

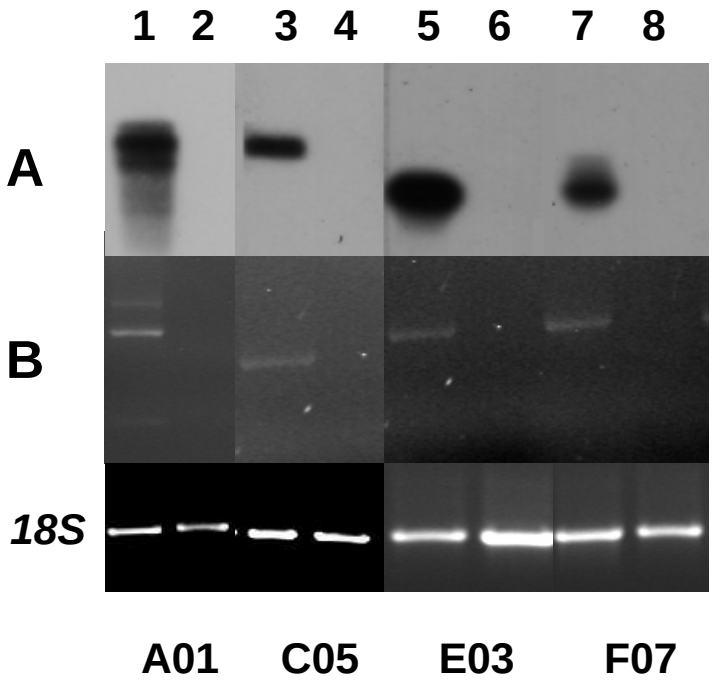

Figure 3-6. Gene expression analysis of differential clones (SSH-NE-PL5-A01, SSH-NE-PL5-C05, SSH-NE-PL5-E03, and SSH-NE-PL5-F07) obtained from the NemaTAM subtracted library. A = Northern blot analysis of differential clones; B = RT-PCR analysis of differential clones; Lane 1, 3, 5, 7 = NemaTAM; Lane 2, 4, 6, 8 = Florunner.

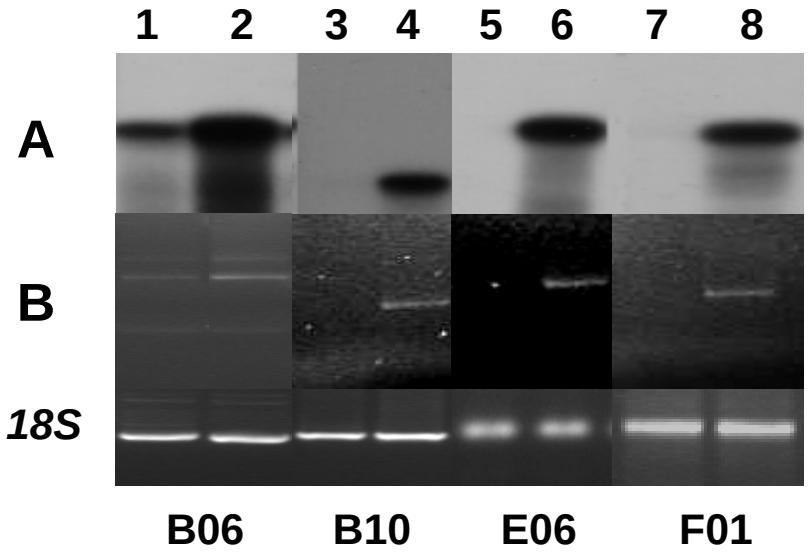

Figure 3-7. Gene expression analysis of differential clones (SSH-FL-PL5-B06, SSH-FL-PL5-B10, SSH-FL-PL5-E06, and SSH-FL-PL5-F01) obtained from the Florunner subtracted library. A = Northern blot analysis of differential clones; B = RT-PCR analysis of differential clones; Lane 1, 3, 5, 7 = NemaTAM; Lane 2, 4, 6, 8 = Florunner.

CHAPTER 4
MOLECULAR MARKER-BASED SELECTION FOR COMBINING ROOT-KNOT NEMATODE RESISTANCE WITH HIGH OLEIC ACID CONTENT IN FLORIDA BREEDING LINES

Introduction

Cultivated peanut (*Arachis hypogaea* L.), is grown in over 110 countries between 40^{o} N and 40^{o} S for both human consumption and as an oil seed crop. It occupies approximately 31% of the total cropped area for oil seeds and accounts for 36% of total oil seed production in the world, ranking fifth among oil seed crops (Marcio et al., 2004). In the United States, peanuts are mostly grown in the south where they have significant economic impact. Consumption of peanut in the United States is about 6 pounds annually per person (Beyer et al., 2001).

Consumers in the United States have become increasingly health conscious with respect to their food intake (Isleib et al., 2004). Consequently, the peanut industry has a greater concern regarding the composition of peanut seed, so as to enhance the marketability of various peanut products. Peanut seed composition is largely a function of lipid chemistry. Peanut contain approximately 50% oil (Cobb and Johnson, 1973). Palmitic acid (16:0), oleic acid (18:1), and linoleic acid (18:2) are the major fatty acids found in peanut seeds and they may comprise > 90% of the total fatty acids (Ahmed and Young, 1982; Norden et al., 1987). Peanuts contain mostly unsaturated fatty acids which are associated with improved cardiovascular health (Paterson et al., 2004). For instance, it has been reported that diets with high monounsaturated fatty acids (i.e. 18:1) and low saturated fatty acids reduce plasma cholesterol levels (Moore and Knauft, 1989), serum cholesterol levels (O'Byrne et al., 1997) and low-density lipoproteins (LDL) in humans (Grundy, 1986; Kris-Etherton et al., 1999). Peanut oil with reduced linoleic acid and high oleic acid content has several advantages, such as enhanced nutritional quality and improved oil stability (St Angelo and Ory, 1973; Grundy, 1986), enhanced shelf life (Moore and Knauft,

1989) decreased risk of rancidity of the roasted peanuts (O'Keefe et al., 1993; Braddock et al., 1995), significant human health advantages (cardiovascular) and enhanced marketability (Andersen and Gorbet, 2002).

Norden et al. (1987) first reported a peanut line, UF435, with 80% oleic and 2% linoleic acid. High oleic peanut varieties, SunOleic 95R (Gorbet and Knauft, 1997) and SunOleic 97R (Gorbet, 2000), were obtained from a cross between the high oleic breeding line UF435 and a component line of 'Sunrunner' (Gorbet and Knauft, 1997). Moore and Knauft (1989) reported that the high oleic acid trait is controlled by two recessive genes ol_1 and ol_2, and if both genes are present, the oleic acid content increases to greater than 70% and linoleic acid content to below 5%. Although high oleic varieties have improved lipid chemistry and are popular among some peanut processors, they are highly susceptible to root-knot nematodes.

In addition to genetic determinants and abiotic factors, peanut seed quality and production are affected by numerous biotic stresses such as insects, fungi, viruses, bacteria and nematodes. Peanut root-knot nematodes are major parasites in all peanut-growing regions of the world. Economic losses due to root-knot nematodes can be substantial in many peanut-producing areas in the United States (e.g. Florida, Georgia, Alabama, Texas and North Carolina). The two major *Meloidogyne* species, *M. arenaria* (peanut root-knot nematode) and *M. hapla* (Northern root-knot nematode), parasitize peanut and are responsible for causing most of the severe yield decreases. The reduction in peanut yield and quality is the result of heavy galling of roots, pegs, and pods following root-knot nematode infection of the root system (Rodriguez-Kabana et al., 1986). Although nematicides like Telone (1, 3 – Dichloropropene) can control root-knot nematodes, they may be harmful to other beneficial organisms in the soil and risky to the applicator. Moreover, nematicides increase production costs and are hazardous to farmers.

Therefore, the development and deployment of root-knot nematode-resistant peanut cultivars in combination with crop rotation is the most desirable alternative to reduce root-knot nematode damage.

However, screening for resistance to root-knot nematode infection within *A. hypogaea* has not identified good resistant sources (Holbrook and Noe, 1992). Fortunately, high levels of resistance were observed in several wild, diploid species (Nelson et al., 1989; Stalker et al., 1987). Two interspecific hybrids, TxAG-6 and TxAG-7, which are highly resistant to root-knot nematodes, as well as major foliar diseases, like leaf spot and rust, were developed by Simpson et al. (1993). TxAG-6 was backcrossed with the cultivar Florunner to develop two new cultivars COAN (Simpson and Starr, 2001) and NemaTAM (Simpson et al., 2003). Although they are resistant to *M. arenaria* race1, these cultivars have reduced yield potential and are low in their oleic/ linoleic fatty acid ratio (NemaTAM is 1.3, Florunner is 1.6; Simpson et al., 2003). With these factors under consideration, breeding efforts were focused on combining root-knot nematode resistance with the high oleic acid trait (80% 18:1) into agronomically superior peanut varieties. This effort should result in the development of peanut cultivars with better qualities for consumers as well as improved efficiency for producers.

Markers such as Random Amplified Polymorphic DNA (RAPD) and Restriction Fragment Length Polymorphism (RFLP) have been used to monitor introgression of wild *Arachis* chromosome segments into cultivated peanut (Garcia et al., 1995). Several RAPD (RKN 229, RKN 410, and RKN 440) and RFLP (R2430E, R2545E, and S1137E) markers have been reported to be linked to root-knot nematode resistance loci in peanut (Burow et al., 1996; Choi et al., 1999). Some of these markers, RKN 229, RKN 410 (Burow et al., 1996) and R2430E (Choi et al., 1999) are linked to the root-knot nematode resistance locus in the breeding lines and

cultivars derived from TxAG-6. These reports indicate that root-knot nematode resistance in these genotypes is inherited as a single dominant gene that was derived from *A. cardenasii* (Dickson and DeWaele, 2005). The above markers were used to select individuals for root-knot nematode resistance from segregating populations in the development of COAN and NemaTAM (Garcia et al., 1996; Church et al., 2000). However, these studies did not examine the use of both RAPD and RFLP markers in the same breeding material to determine which would be more efficient.

Peanut cultivars that are high oleic and resistant to root-knot nematode are currently not available. Consequently, an objective of the University of Florida peanut breeding program was to develop peanut breeding lines that have root-knot nematode (*M. arenaria*) resistance along with high oleic acid content. The interspecific hybrids, TxAG-6 and TxAG-7, which were used in the development of COAN, were used as parents for root-knot nematode resistance in Florida breeding material. Populations were initially selected for resistance to leaf spot, tomato spotted wilt virus, and agronomic traits, but not for root-knot nematode resistance. As mentioned earlier, there were reports identifying molecular markers linked to a root-knot nematode resistance locus, but very limited data were available to compare the efficiency of marker-assisted selection procedures to other selection techniques. In the present study, the utility of marker-assisted selection using a previously identified SCAR marker, Z3/265 (Garcia et al., 1996) and the RFLP marker R2430E (Church et al., 2000) is reported for combining root-knot nematode resistance with high oleic acid content in peanut.

Materials and Methods

Plant Materials

Experiment 1

Six University of Florida breeding lines (F1334; F79x4-6; F94x30-8-2-1-b3; F94x30-8-3-1-b2; F94x30-8-2-2-b3; F94x30-5-2-3-3-b3), and the Texas A&M University breeding line, TP301-1-8, were planted in field plots in 2001 at the Plant Science Unit, Teaching and Research Center, University of Florida, Gainesville, FL. The field plots were two 5.0 m long rows with 90 cm inter rows with four replications. The field was infested with root-knot nematodes, *M. arenaria* race 1 based on established protocols for reproduction (Starr et al, 1995). During the development of the F94x30 lines, the interspecific hybrid TxAG-6 was used as a parent. Peanut cultivars Florunner and COAN were included in this test as root-knot nematode susceptible and resistant controls, respectively. Ten plants were labeled in each plot. Young leaves were collected from those plants, frozen in liquid N_2, and stored at –80 ^{o}C until use. Root-knot nematode reproduction was measured on the same 10 plants previously labeled in each replication and used for molecular marker analysis, as described below.

Experiment 2

Crosses were made in 2002 by the University of Florida breeding program involving the root-knot nematode resistant parent COAN and root-knot nematode susceptible parents HULL (Gorbet, 2007a), ANorden (Gorbet, 2007b), F89XOL14-1-4-1-1-1-2 and 92x0L19-8-1-1 (Table 4-1). A total of 740 seed from F_1 plants of these crosses were assayed for the presence of the SCAR marker, Z3/265, as described below. All seed from these crosses (F_2) that had the marker were subsequently planted in 2003 (F_2 generation) at the Plant Science Unit, Teaching and Research Center, University of Florida, Gainesville, FL. Leaves from 50 F_2 plants were selected based on their desirable agronomic traits and subjected to RFLP analysis and seed were analyzed

for oleic acid, as described below, before their advancement to the F_3 generation. Seeds from F_2 plants and their parents were planted in a root-knot nematode-infested field in 2004, at the Plant Science Unit, Teaching and Research Center, University of Florida, Gainesville, FL as described for experiment one above in a Randomized Complete Block Design (RCBD) with four replications. Each replication consisted of 13 rows that were 3.6 m long with 1 m between rows and 30 cm between plants in rows, giving a total number of approximately 28 F_3 plants per plot. Each row consisted of four lines separated by 1 m. Plot size was 11 m x 12 m.

Root-knot Nematode Resistance Test

One hundred days after planting, plants were harvested and evaluated for root-knot nematode resistance. The resistance to *M. arenaria* race 1 was measured based on the number of egg masses and galls present on peanut roots and pegs. Plants were harvested and the soil was washed from the roots with tap water. Roots were then placed into 3000 ml beakers containing approximately 900 ml of 0.05% Phloxine B / L solution for 3 - 5 min (Daykin and Hussey, 1985). Root gall and egg mass ratings were assigned based on the scale described in Table 4-2. Briefly, each plant was rated according to the number of egg masses and galls found on roots, pegs and pods. A plant given a rating of 1 (no galls or egg masses on roots, pegs and pods) was considered highly resistant, a plant rated as 2 (1-10 egg masses and/or galls on roots and less than 10 egg masses and/or galls on pegs and pods) was considered resistant, a rating of 3 (11-100 egg masses and/or galls on roots and between 10-50 egg masses and/or galls on pegs and pods) indicated that the plants were susceptible and a plant rated as 4 (> 100 egg masses and/or galls on roots and > 50 egg masses and/or galls on pegs and pods) was considered highly susceptible. Root-knot nematode count data were subjected to analysis of variance and the treatment means were compared by Tukey's studentized range test (SAS Institute, 1985). Statistical difference was assumed at $P = 0.05$.

DNA Extraction from Seeds and Leaves

Total genomic DNA was extracted from seed according to Chenault et al. (2007). Briefly, a small section (0.02 g) of seed was removed taking care not to damage the embryo for planting purposes, and placed in a 1.5 ml eppendorf containing 400 µl of extraction buffer (100 mM Tris pH 8.0; 50 mM EDTA pH 8.0; 500 mM NaCl, and 10 mM mercaptoethanol added fresh) at 60 ^{o}C. The seed samples were ground into a milky-white solution using pestle and mortar, and transferred to a fresh tube containing 200 µl of 20% SDS for 10 min. This was followed by the addition of prechilled 5 M potassium acetate (5 ml), two phenol/chloroform/isoamyl (25:24:1) alcohol extractions, and a final precipitation with ice cold isopropanol. DNA was pelleted by centrifugation at 23000 × g for 20 min at 4 ^{o}C. After air drying, and rinsing in 70% ethanol, the pellet was resuspended in buffer (10 mM Tris (pH 8.0), 1 mM EDTA).

Genomic DNA extraction from leaves was performed according to the Dellaporta method (1983) with the following modifications. Briefly, DNA was extracted from 1.2 g of leaf tissue with 15 ml extraction buffer (100 mM Tris-HCI, pH 8.0, 50 mM EDTA, and 500 mM NaCI) at 60 ^{o}C followed by prechilled 5 M potassium acetate (5 ml) treatment, two chloroform/isoamyl alcohol extractions and a final precipitation with ice cold isopropanol. DNA was pelleted by centrifugation at 20,000 x g for 20 min at 4 ^{o}C and rinsed twice with 70% ethanol. After air drying, the DNA was re-suspended in water followed by RNAase (20 pg/ml) treatment and centrifugation at 20,000 x g for 15 min at 4 ^{o}C. The DNA pellet was re-suspended in TE buffer and stored at –20 ^{o}C.

SCAR Analysis

Forward primer, SCZ3-FO1 5'-CAGCACCGCAGCATAAAAAC-3' and reverse primer, SCZ3-RO2 5'-CAGCACCGCACACATTCTGG-3' were used to amplify a SCAR marker with an expected size of 310 bp (Garcia et al., 1996). PCR reactions were performed with a MJ

Research thermocycler (Waltham, MA) under the following conditions: 94 ^{o}C for 5 min, 30 cycles at 94 ^{o}C for 1 min, 60 ^{o}C for 1 min, 72 ^{o}C for 2 min, and a final extension at 72 ^{o}C for 3 min. The root-knot nematode susceptible cultivar, Florunner, was used as a negative control and the root-knot nematode resistant cultivar, NemaTAM was used as a positive control for all experiments.

Oleic Acid Analysis

Seeds from fifty F_2 plants that were positive for the SCAR marker and agronomically superior were then analyzed by George D. Person (laboratory technician, University of Florida) using fourier transform infrared (FTIR) spectrometer (Nexus 870 FTIR, Thermo Nicolet, Madison, WI) for oleic acid content. Seeds were then characterized as high oleic (> 75% oleic acid content), medium oleic (60 – 75% oleic acid), and low oleic (< 60% oleic acid). These seeds were then planted at the University of Florida, Pine Acres Research Station, Citra, FL. Leaf tissue was collected from 50 of the resulting plants. The tissue was quick frozen in liquid nitrogen and stored at –80 ^{o}C until use for RFLP analysis.

RFLP Analysis

Peanut genomic DNA (20 µg) was digested overnight with EcoRI according to the manufacturer's instructions (New England Biolabs, Beverly, MA). Digested DNA was subjected to electrophoresis for 16 h using 0.8% agarose gels and transferred onto Hybond N+ membranes (Amersham, Arlington Heigts, IL) by capillary blotting (Southern, 1975) and UV cross linked for 3 min at 70,000 micro-joules/cm^2. The RFLP probe, R2430E, was kindly provided by Dr. Gregory T. Church (Texas A&M University). Probe DNA (50 ng) was labeled with α-P^{32} dCTP by random primer extension (Feinberg and Vogeltein, 1983). Pre-hybridization and hybridization were performed at 65 ^{o}C with 7% SDS and denatured salmon sperm DNA (Church and Gilbert, 1984). Samples were washed three times for 20 min each at 65 ^{o}C with 0.5X SSC and 0.1% SDS.

Hybridized blots were autoradiographed using X-ray film (Kodak XAR-5) and two intensifying screens at –70 ^{o}C for three to 10 d.

Results

The root-knot nematode resistant and susceptible alleles detected using R2430E were distinct and easy to score for lines F1334, F79x4-6, F94x30-8-2-1-b3, F94x30-8-3-1-b2, F94x30-8-2-2-b3 and F94x30-5-2-3-3-b3. All lines with a susceptible RFLP genotype (Fig. 4-1) had a susceptible phenotype based on root-knot nematode reproduction as described in Table 4-2. Field results showed that the gall and egg mass index for the root-knot nematode resistant cultivar COAN was 1, whereas the mean gall and egg mass index on roots and pods of the susceptible cultivar Florunner was 3.2 (Table 4-3). Reproduction of *M. arenaria* on each of the lines as measured by the number of galls and egg masses on roots ranged from 3.0 to 3.9 with greater than 11% egg masses on pods and pegs which was more than the reproduction found on Florunner ($P < 0.001$) (Table 4-3). No resistance to *M. arenaria* was observed in any of the six Florida breeding lines.

Consequently, to incorporate root-knot nematode resistance into the Florida germplasm along with the high oleic trait, several crosses involving the root-knot nematode resistant cultivar COAN and susceptible high oleic cultivars HULL (Gorbet, 2007a), ANorden (Gorbet, 2007b), UF 98326 and lines F89xOL14-1-4-1-1-1-2, and 92x0L19-8-1-1 (Table 4-3) were made and the seed from F_1 plants was screened for root-knot nematode resistance with the SCAR marker Z3/265 (Garcia et al., 1996). This marker was chosen over the RFLP marker due to its ease for screening.

A total of 740 seed from F_1 plants were screened for Z3/265 and 193 were positive for the presence of the marker (Table 4-4; Fig. 4-2). All the seeds from F_1 plants positive for the presence of Z3/265 were subsequently advanced to the F_2 generation. Leaves from 50 plants

(Table 4-5) that were agronomically sound and disease free were collected and subjected to oleic acid and RFLP analysis with the main objectives of determining a relationship between the presence of the SCAR marker and RFLP marker as a means to test for reliability and zygosity, and for further advancing to the F_3 generation. Thirty of the 50 plants were high oleic (> 75%), 9 were medium oleic (65 - 70%) and 11 (< 60%) were low oleic (Table 4-5). There was no relationship between root-knot nematode resistance and the high oleic acid trait (Table 4-5).

Using R2430E, individuals plants were scored as homozygous for resistance (RR) if only the band associated with resistance was present; heterozygous for resistance (RS) if both the band for resistance and susceptibility were present, and susceptible (SS) if the band associated with susceptibility was present (Fig. 4-3). Five (10%) out of 50 F_2 selections were homozygous for the susceptible marker and six (12%) were homozygous for the resistance marker, with the remainder (74%) being heterozygous (Table 4-5). Three selections homozygous for the S-allele were susceptible and the remaining heterozygous and R-allele homozygotes were resistant (Table 4-5).

Finally, these 50 F_2 selections were planted in a field highly infested with root-knot nematodes (*M. arenaria* race 1). Resistance to *M. arenaria* race 1 was measured based on number of galls present on peanut roots and pegs (Table 4-2). Forty-four selections (88%) were found to be resistant and three selections (18%) were susceptible (Table 4-5). For the resistant control NemaTAM, the mean peg and pod gall index was 1 and root gall index was 1, whereas for the susceptible control Florunner, the mean peg and pod gall index was 1 and root gall index was 3 (Table 4-5). Reproduction of *M. arenaria* on each of the selections as measured by the peg and pod gall index and root gall index ranged from 0.0 to 1 and 1 to 3 respectively (Table 4-5).

Reproduction of *M. arenaria* on each of the selections as measured by the number of galls on roots, pegs and pods ranged from 0 to greater than 100.

Discussion

Results of RFLP analysis of six Florida breeding lines indicated that none of this Florida breeding material inherited the locus for root-knot nematode resistance from TxAG-6. A possible explanation for this result is that the root-knot nematode resistance gene was lost in segregating generations subsequent to the F_2 because no selection for root-knot nematode resistance was used to advance these materials until the screening employed in this study. Also TxAG-6 was heterozygous for the resistance and the gene may not have been transferred in the cross. This result prompted the use of marker-assisted selection for screening the earlier generations for root-knot nematode resistance with the main objective of introgression of root-knot nematode resistance into lines carrying the high oleic acid trait.

The SCAR marker Z3/265, and the RFLP R2430E analyses of filial generations (F_1 and F_2) of crosses involving the root-knot nematode resistant cultivar COAN and the susceptible cultivars HULL (Gorbet, 2007a), and ANorden (Gorbet, 2007b) and lines F89xOL14-1-4-1-1-1-2 and 92x0L19-8-1-1, indicated co-segregation of these markers. Based on this, it can be hypothesized that both the markers might be linked to the same root-knot nematode resistance gene.

Resistance in some selections (Table 4-5), despite the absence of the R2430E marker was possible because R2430E is 4.2 cM away from the resistance locus, and this indicates a possible recombination of 4% between marker and root-knot nematode resistance (Choi et al., 1999; Church et al., 2000). RFLP data were somewhat more reliable than SCAR marker data as there was strong relationship (90% of F_2 selections that were RFLP marker positive were root-knot nematode *M. arenaria* resistant) between the presence of the RFLP marker and root-knot

nematode resistance as measured in the field (Table 4-5). Moreover, the RFLP marker allowed for the selection of homozygous individuals for root-knot nematode resistance (Church et al., 2000). However, using the RFLP marker resulted in a higher frequency of lost data due to several factors such as insufficient DNA availability, incomplete digestion of DNA, and poor hybridization (Church et al., 2000). In addition, RFLP analysis is more cumbersome and costly than SCAR analysis. SCAR marker analysis is simple, rapid and cost effective when it comes to screening large numbers of breeding lines. The primary drawback of SCAR markers is that they are dominant and do not permit the scoring of heterozygous individuals. However, the SCAR marker Z3/265 derived form *A. cardenasii* is linked at 10 $\pm$ 2.5 cM and 14 $\pm$ 2.9 cM from the root-knot nematode resistance genes *Mag* and *Mae,* respectively (Garcia et al., 1996), which indicates a possible recombination frequency of 10 to 14%. This high rate of recombination might result in false positives (Chu et al., 2007). Recently, Chu et al. (2007) developed a dominant marker 197/909, which is about 5.8 cM from the root-knot nematode resistance gene. This still leaves room for a recombination frequency of 5.8%. Therefore, a more tightly linked SCAR marker would be desirable. Also mature peanut leaf has high amounts of phenolic compounds and polysaccharides; these compounds interfere with *Taq* polymerase (Sharma et al., 2000; Chenault et al., 2007) affecting the reproducibility of Z3/265 marker. So in order to overcome this problem peanut seed DNA was used in our study to perform SCAR marker analysis.

According to the root gall index of the F_3 generation (Table 4-5), there is a discreet reaction with respect to root-knot nematode resistance, but the peg and pod gall index is not as uniform. This can be explained by the recent report that the resistance gene in COAN has two

major effects when compared to susceptible cultivars, penetration and further development after penetration (Bendezu and Starr, 2003).

Marker-assisted selection helps in identifying those individuals in which desirable recombination events have occurred during backcrossing of the nematode resistance gene into elite peanut cultivars. In addition, marker-assisted selection has the potential to significantly reduce the cost associated with field planting, maintenance and field trails. Furthermore, in this study marker assisted selection allowed for peanut genotypes to be screened without relying on inoculation tests in advanced generations, which in turn reduces the time for developing root-knot nematode resistant peanut cultivars.

Table 4-1. Sample number representation, parents involved and crosses made for seed from F_1 plants.

Sample #	Cross #	Parents
1	01 X 14 -1	Hull X COAN
2	01 X 14 -1	Hull X COAN
3	01 X 14 -1	Hull X COAN
4	01 X 14 -1	Hull X COAN
5	01 X 14 -1	Hull X COAN
6	01 X 22 -1	COAN X ANorden
7	01 X 22 -1	COAN X ANorden
8	01 X 22 -1	COAN X ANorden
9	01 X 22 -1	COAN X ANorden
10	01 X 22 -1	COAN X ANorden
11	01 X 22 -2	COAN X ANorden
12	01 X 22 -2	COAN X ANorden
13	01 X 22 -2	COAN X ANorden
14	01 X 22 -2	COAN X ANorden
15	01 X 22 -2	COAN X ANorden
16	01 X 22 -3	COAN X ANorden
17	01 X 22 -3	COAN X ANorden
18	01 X 22 -3	COAN X ANorden
19	01 X 22 -3	COAN X ANorden
20	01 X 22 -3	COAN X ANorden
21	01 X 23 -1	COAN X UF 98326
22	01 X 23 -1	COAN X UF 98326
23	01 X 23 -1	COAN X UF 98326
24	01 X 23 -1	COAN X UF 98326
25	01 X 23 -1	COAN X UF 98326
26	01 X 24 -1	COAN X F89xOL14-1-4-1-1-1-2
27	01 X 24 -1	COAN X F89xOL14-1-4-1-1-1-2
28	01 X 24 -1	COAN X F89xOL14-1-4-1-1-1-2
29	01 X 24 -1	COAN X F89xOL14-1-4-1-1-1-2
30	01 X 24 -1	COAN X F89xOL14-1-4-1-1-1-2
31	01 X 24 -2	COAN X F89xOL14-1-4-1-1-1-2
32	01 X 24 -2	COAN X F89xOL14-1-4-1-1-1-2
33	01 X 24 -2	COAN X F89xOL14-1-4-1-1-1-2
34	01 X 24 -2	COAN X F89xOL14-1-4-1-1-1-2
35	01 X 24 -2	COAN X F89xOL14-1-4-1-1-1-2
36	01 X 26 -1	COAN X 92x0L19-8-1-1
37	01 X 26 -1	COAN X 92x0L19-8-1-1
38	01 X 26 -1	COAN X 92x0L19-8-1-1
39	01 X 26 -1	COAN X 92x0L19-8-1-1
40	01 X 26 -1	COAN X 92x0L19-8-1-1

Table 4-1 (Continued)		
Sample #	Cross #	Parents
41	01 x 26 -2	COAN X 92x0L19-8-1-1
42	01 x 26 -2	COAN X 92x0L19-8-1-1
43	01 x 26 -2	COAN X 92x0L19-8-1-1
44	01 x 26 -2	COAN X 92x0L19-8-1-1
45	01 x 26 -2	COAN X 92x0L19-8-1-1
46	01 x 26 -3	COAN X 92x0L19-8-1-1
47	01 x 26 -3	COAN X 92x0L19-8-1-1
48	01 x 26 -3	COAN X 92x0L19-8-1-1
49	01 x 26 -3	COAN X 92x0L19-8-1-1
50	01 x 26 -3	COAN X 92x0L19-8-1-1
51	Florunner	Susceptible Control
52	NemaTAM	Resistant Control

Table 4-2. Gall and egg mass index scale (1-4) used to evaluate peanut genotypes for resistance to *Meloidogyne arenaria* (Dr. D.W. Dickson, Pers. Comm.).

Grade	No of galls or egg masses on roots	No of galls or egg masses on pegs and pods	Reaction
1	0	0	Highly Resistant
2	1 – 10	<10	Resistant
3	11- 100	10 – 50	Susceptible
4	> 100	> 50	Highly Susceptible

Table 4-3. Field reaction of peanut breeding lines to *Meloidogyne arenaria* evaluated at the Plant Science Unit – University of Florida, FL., 2001.

Genotypes[a]	Gall and egg mass index (Average/STDV)[b]	Egg mass on pods and pegs (%)	Classification[c]
F1334	3.90/0.23 [a]	11-50	S
F94x30-8-2-2-b3	3.90/0.31 [a]	11-50	S
F94x30-5-2-3-3-b3	3.80/0.41 [a]	11-50	S
F94x30-8-3-1-b2	3.70/0.57 [a]	> 50	HS
F94x4-6	3.60/0.50 [a]	11-50	S
F94x30-8-2-1-b3	3.60/0.50 [a]	> 50	HS
Florunner	3.20/0.36 [b]	> 50	HS
TP301-1-8	1.00/0.00 [c]	0	R
COAN	1.00/0.00 [c]	0	R

[a]T301-1-8 = resistant breeding line; COAN = resistant genotype; Florunner = susceptible genotype. [c]HS = highly susceptible, R = resistant, S =susceptible.
[b]Means within a column followed by the same letter are not statistically different at P = 0.05 based on Tukey's studentized range test.

Table 4-4. Crosses tested using the SCAR marker for root-knot nematode resistance.

Cross	Total No. of seed tested	No. of seed positive for the SCAR marker	% Positive
HullXCOAN	137	35	26
COANXANorden	223	19	8.5
COANXUF98326	78	39	50
COANXF89/OL14-1-4-1-1-1	184	34	18
COANX920L19-8-1-1	112	66	59
Total	740	193	26

Table 4-5. Comparison of oleic acid content, dominant SCAR marker Z3/265 and co-dominant RFLP marker R2430E data with root-knot nematode field reaction.

Sample #	Seed oleic acid content[a]	Z3/265 Seed marker data[b]	R2430E leaf Southern data[c]	Peg and pod gall index	Root gall index	Root-knot nematode field Reaction
1	H	+	-	1	3	Susceptible
2	H	+	+ -	1	1	Resistant
3	H	+	+ -	1	1	Resistant
4	H	+	+ -	1	1	Resistant
5	H	+	-	1	2	Resistant
6	H	+	+ -	1	1	Resistant
7	H	+	+ -	1	1	Resistant
8	H	+	+ -	1	1	Resistant
9	H	+	+ -	1	1	Resistant
10	H	+	+ -	0	0	0
11	H	+	+ -	1	2	Resistant
12	H	+	+ -	1	1	Resistant
13	H	+	+	1	1	Resistant
14	H	+	+ -	1	2	Resistant
15	M	+	+ -	1	1	Resistant
16	M	+	+ -	1	1	Resistant
17	L	+	-	1	3	Susceptible
18	L	+	+	1	1	Resistant
19	L	+	+ -	1	1	Resistant
20	L	+	+ -	1	1	Resistant
21	H	+	0	1	1	Resistant
22	H	+	+ -	1	1	Resistant
23	H	+	+ -	1	1	Resistant
24	H	+	+ -	1	1	Resistant
25	H	+	+ -	1	2	Resistant
26	H	+	+	1	1	Resistant
27	L	+	+ -	1	2	Resistant

Table 4-5 (Continued)

Sample #	Seed oleic acid content[a]	Z3/265 Seed marker data[b]	R2430E leaf Southern data[c]	Peg and pod gall index	Root gall index	Root-knot nematode field Reaction
28	L	+	+	1	1	Resistant
29	L	+	+ -	1	1	Resistant
30	L	+	+ -	1	2	Resistant
31	H	+	+ -	1	1	Resistant
32	H	+	+ -	1	1	Resistant
33	M	+	+ -	0	0	0
34	M	+	+ -	1	1	Resistant
35	M	+	+ -	1	1	Resistant
36	M	+	+ -	1	1	Resistant
37	M	+	+ -	1	1	Resistant
38	L	+	+ -	1	2	Resistant
39	L	+	+ -	1	1	Resistant
40	L	+	+ -	0	0	0
41	H	+	+ -	1	1	Resistant
42	H	+	+	1	1	Resistant
43	H	+	+ -	1	1	Resistant
44	H	+	+ -	1	1	Resistant
45	M	+	+ -	1	1	Resistant
46	H	+	0	1	1	Resistant
47	H	+	+	1	1	Resistant
48	H	+	-	1	3	Susceptible
49	H	+	+ -	1	1	Resistant
50	M	+	-	1	1	Resistant
51	L	-	-	1	3	Susceptible
52	M	+	+	1	1	Resistant

[a]H = High oleic, M = Medium oleic, L = Low oleic

[b]Marker present (+), Marker absent (-)

[c]Heterozygous positive (+ -), Homozygous positive (+), Homozygous negative (-), No results (0); 0= No data

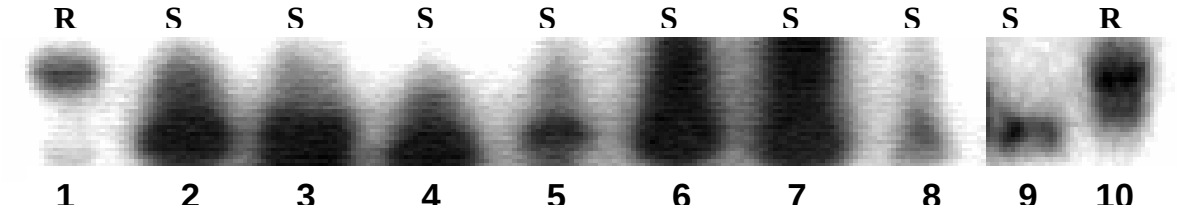

Figure 4-1. RFLP locus R2430E linked to resistance to *Meloidogyne arenaria* in peanut breeding lines. R = resistant and S = susceptible alleles. Lane 1 is the root-knot nematode resistant cultivar COAN; lane 2 is the susceptible cultivar Florunner; Lanes 3 to 9 are the breeding lines F1334; F94x4-6; F9430-8-2-1-b3; F94x30-8-3-1-b2; F94x30-8-2-2-b3; F94x30-5-2-3-3-b3, F94x30-5-2-3-3-b3, respectively; lane 10 is the root-knot nematode resistant breeding line T301-1-8.

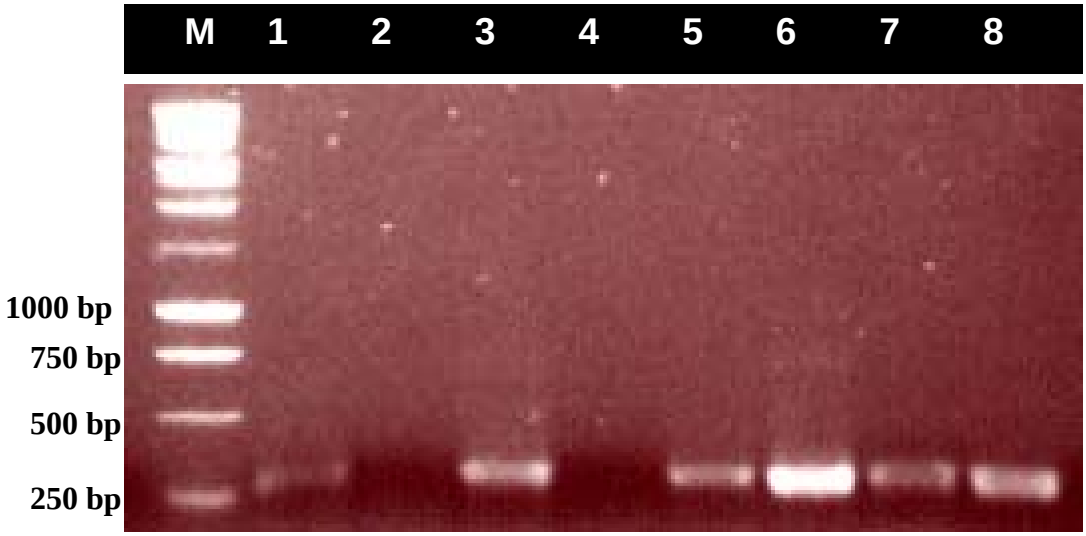

Figure 4-2. SCAR marker analysis. M = 1kb Ladder. Amplicons of Z3/265 marker: Lane 1 = COAN, Lane 2 = Florunner, Lanes 3-8 = F_2 seed tested.

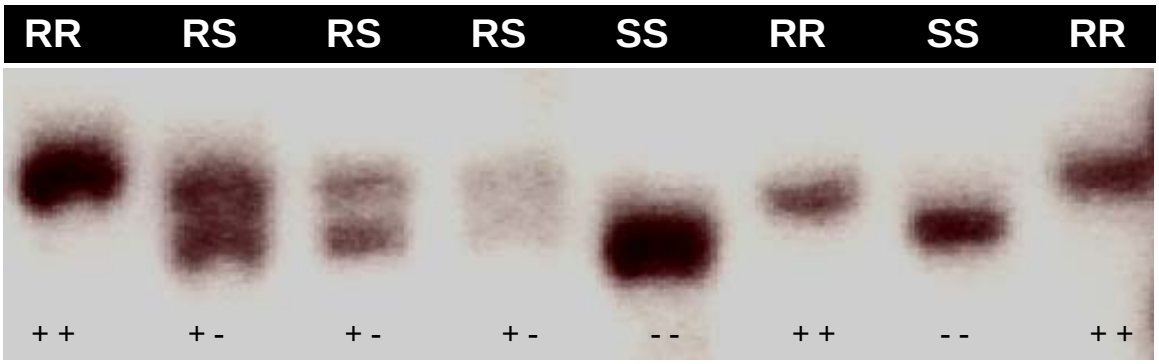

Figure 4-3. RFLP marker analysis. RR = homozygous resistant (++); SS = homozygous susceptible (--); RS = heterozygous resistant (+-).

CHAPTER 5
CLONING AND CHARACTERIZATION OF *R*-GENES IN PEANUT (*ARACHIS HYPOGAEA* L.)

Introduction

Peanut (*Arachis hypogaea* L.) production is affected by many biotic constraints such as, insects, microbial pathogens and root-knot nematodes. In the approximately 35% of crop losses that occurs annually, 12% is due to diseases, 7% is due to insects, and 11% is due to nematodes. Root-knot nematodes cause significant yield losses in many peanut-producing areas (e.g. Florida, Georgia, Alabama, Texas and North Carolina). In the United States, estimated annual monetary losses due to nematodes in peanut is $102 million (Sasser, 1990). As much as a 30% decrease in peanut yield potential has been observed due to *M. arenaria* alone (Nelson et. al., 1990).

Root-knot nematode infection of the root system of peanut plants results in heavy galling of roots, pegs, and pods. This in turn reduces peanut quality and yield (Rodriguez-Kabana et al., 1986). Root-knot nematodes have a broad host range so controlling them by crop rotation is difficult. Although nematicides are effective, their continued usage is uncertain due to environmental hazards and economic concerns (Chen et al., 1996). Moreover, the application of some nematicides has been abandoned in some areas because of their associated toxicity to farmers (Jung et al., 1998). Consequently, the best method of controlling the root-knot nematode, *M. arenaria*, is by deployment of root-knot nematode resistant peanut cultivars.

Several resistance genes (*R* genes) against nematodes have been cloned and mapped. Other than the first nematode *R* gene identified, *Hs1pro-1*, all the other nematode *R* genes share structural homology with other plant disease resistance genes (Baker et al., 1997; Cai et al., 1997; Ellis and Jones, 1998; Williamson, 1998). Most of these nematode *R* genes code for members of a family characterized by a nucleotide-binding site (NBS) and leucine-rich repeats (LRRs) (Lagudah et al., 1997; Milligan et al., 1998). For example, the potato cyst nematode

resistance gene, *Gpa2*, encodes a product with NBS and LRR domains (Van der Vossen et al., 2000) and shows high homology with the virus resistance gene *Rx* (Davis et al., 2000). Several studies have also shown that this resistance against potato virus X operates on a gene-for-gene basis in which the *R* gene encodes a putative receptor that recognizes the viral coat protein (Bendahmane et al., 1999). Functional and genomic studies of the *R* genes above revealed that the NBS-LRR, LRR, and LRR-Kinase superfamilies are highly conserved and ubiquitous in plants (Young, 2000).

Breeding for root-knot nematode resistance in peanut based on inoculation and phenotypic selection is a challenging process due to irregular distribution of root-knot nematode populations in the field and several other field constraints. Currently, knowledge of peanut resistance genes (*R*) is limited and their use for peanut genetic improvement lags behind most crop plants. A better understanding of the molecular and genetic basis of nematode resistance (*R*) genes would enhance the effective use of *R* genes as molecular markers in peanut breeding for nematode resistance. However, effective use of these *R* genes as molecular markers first requires isolation and characterization of these genes.

To date large scale genome wide analysis of disease resistance gene-like sequences in *Arachis* species has been conducted by Bertioli et al. (2003) and Yuksel et al. (2005). Seventy-eight resistance gene homologues (RGHs) were generated with degenerate primers targeting NBS region from *A. hypogaea* var. Tatu and four wild relatives (*A. duranensis, A. cardenasii, A. stenosperma*, and *A. simpsonii*) (Bertioli et al., 2003). Yuksel et al. (2005) designed primers targeting consensus sequences of various classes of resistance genes and isolated 234 resistance gene analogs (RGAs). Their phylogenetic analysis of these RGAs indicated their evolutionary proximity to RGAs from other legume species, including *Glycine max*, *Lotus japonicus*,

Medicago truncatula, and *Phaseolus vulgaris*. In the current study, the objective was to clone *R* genes from the root-knot nematode resistant cultivar, NemaTAM, which may confer resistance to the root-knot nematode, *M. arenaria* race 1, using degenerate primers for conserved NBS-LRR domains reported to be associated with resistance genes (Donald et al, 2002).

Materials and Methods

Plant Materials

Individual plants of the root-knot nematode resistant cultivar NemaTAM were grown in clay pots containing pasteurized soil (Arredondo and Sparr type; sand 95%, silt 3%, clay 2% and organic matter 1.5%; pH 6.5) for leaf genomic DNA extraction and PCR amplification of RGAs. Thirty days after planting, genomic DNA was extracted from young leaves of NemaTAM. Similarly, for RT-PCR and gene expression studies, total RNA was extracted from roots of individual plants of NemaTAM and the root-knot nematode susceptible cultivar Florunner grown in clay pots containing pasteurized soil (Arredondo and Sparr type; sand 95%, silt 3%, clay 2% and organic matter 1.5%; pH 6.5). For both experiments, all of the pots were kept in a greenhouse at 27-32 ^{o}C. They were watered daily and fertilized (Miracle-Gro, Marysville, OH) once a week with N-P-K (24-8-16). For RT-PCR / gene expression studies, 10 days after planting, each pot was inoculated with a suspension of 5000 eggs of *M. arenaria* race 1 which had been cultured on tomato (*Solanum esculentum* Mill. cv. Rutgers). The eggs were pipetted into three holes (0.6-cm diameter x 4-cm depth) made close to the roots, but distributed at equal distance from the base of the plants. Root-knot nematode inoculum was prepared using the NaOCl method as described in Hussey and Barker (1973). Total RNA was isolated from roots of NemaTAM and Florunner at five time points: 0, 12, 24, 48, and 72 h after inoculation/infection with *M. arenaria* race 1.

Genomic DNA Extraction

For genomic DNA extraction, leaves stored at –80 ^{o}C were ground and treated according to the Dellaporta method (1983) with the following modifications. Briefly, DNA was extracted from 1.2 g of leaf tissue with 15 ml extraction buffer (100 mM Tris-HCI, pH 8.0, 50 mM EDTA, and 500 mM NaCI) at 60 ^{o}C followed by prechilled 5 M potassium acetate (5 ml) treatment and two chloroform/isoamyl alcohol extractions and final precipitation with ice cold isopropanol. DNA was pelleted by centrifugation at 20,000 x g for 20 min at 4 ^{o}C and rinsed twice with 70% ethanol. After air drying, the DNA was re-suspended in water followed by RNAase (20 pg/ml) treatment and centrifugation at 20,000 x g for 15min at 4 ^{o}C. The DNA pellet was re-suspended in TE buffer and stored at –20 ^{o}C. Each sample yielded 30-50 µg of DNA. The DNA extracted was utilized for amplifying RGAs as described below.

RNA Extraction

Total RNA for gene expression studies was isolated from root samples using the Guanidine–HCl method as described by Logemann et al. (1987) with slight modifications. Roots (3g) were ground in liquid nitrogen, then 15 ml of 6M Guanidine buffer and 15 ml of phenol/chloroform/isoamyl alcohol (PCI; 25:24:1) were added, and the mixture was shaken for 20 min and centrifuged for 20 min at the rate of 20, 000 x g to separate the phases. The supernatant was gently removed and 1.5 vol of absolute ethanol and 0.3 vol of 1M acetic acid (pre-cooled at 4 ^{o}C) were added and the RNA was precipitated overnight at – 20 ^{o}C. The pellet obtained after centrifugation at the rate of 20, 000 x g was resuspended in 5 ml of DEPC-treated water and precipitated by incubation for 1 h at – 20 ^{o}C after the addition of 300 µl of 4M LiCl and 5 ml of cold 100% ethanol. After washing the pellet with 10 ml of cold 70% ethanol, the RNA was air dried, resuspended in 1 ml of distilled sterile water and stored at – 80 ^{o}C.

RT-PCR

Reverse transcription of total RNA was carried out using iScript reverse transcriptase (Bio-Rad, Hercules, CA) with oligo-dT as the primer, according to the manufacturer's instructions. First-strand cDNA from 25 ng total RNA was used in each PCR reaction with the following primer combinations: forward F5'-atctgaaagcatgggtttgc-3' and reverse R5'-gctgctctcccatttgattc-3' for *NRP3D-4*; and for 18S control forward F 5'- aacggctaccaccacatccaaggaaggc-3' and reverse R 5'- gcgcgtgcggcccagaacatctaag-3'. PCR was carried out in a MJ-100 thermocycler (MJ Research, Watertown, MA) with the following program: 94 ^{o}C for 1min, followed by 25 or 30 cycles at 94 ^{o}C for 15 sec, 59 ^{o}C for 20 sec, 72 ^{o}C for 15 sec and a final extension at 72 ^{o}C for 1 min. PCR products were loaded onto a 1% agarose gel and run in 1X TBE containing ethidium bromide at 75 V for 3 hr.

Polymerase Chain Reaction

Amplification of RGAs was performed in 20 µl reactions containing 15-20 ng/µl of NemaTAM DNA, 10 pmole/µl of degenerate primers P1B-fwd, P3D-rev, P3A-rev, P1A-fwd and P3D-rev and RNBS-D-rev (Table 5-1; Bertioli et al., 2003), 2 mM of dNTPs, 10 mM $MgCl_2$ and 10 x buffer and Taq polymerase (1u/ µl) (Invitrogen, Carlsbad, California). Annealing temperatures for each reaction were optimized using a Gradient Cycler (MJ Research PTC-200, Watertown, MA). The annealing temperature that yielded a sufficient amount of PCR product at the desired range was selected. The following primer combinations were used for PCR: LM638 with P3D-rev, P1B-fwd with P3D-rev, P1A-fwd with P3D-rev, P1B-fwd with P3A-rev, LM638 with P3A-rev, and LM638 with RNBS-D-rev (Bertioli et al., 2003). PCR was carried out in a MJ-100 thermocycler (MJ Research, Watertown, MA) with the following program: 5 min at 94 ^{o}C, followed by 30 cycles of 1 min 94 ^{o}C, 1 min at 50 ^{o}C, 2 min at 72 ^{o}C and final extension of 5

min at 72 ^{o}C. PCR products were run on a 1% agarose gels containing ethidium bromide (EtBr-0.5µl of 10 mg/ml) in 1x TBE at 75 V for 3 hr for visualization.

Cloning and Transformation

PCR products resolved by electrophoresis on a 1% agarose gel in 1x TBE buffer were excised. Fragments thus obtained were purified using QIA quick gel extraction columns according to the manufacturer's instructions (Qiagen, Valencia, California). Purified PCR fragments were ligated into pGEM using the pGEM-T Easy cloning kit and propagated in *Escherichia coli* DH5-α cells according to the manufacturer's instructions (Promega, Madison, Wisconsin). Transformed cells were subsequently grown with constant shaking at 300 rpm in 1 ml of SOC medium (2% tryptone, 0.5% yeast extract, 8.5 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 20 mM glucose) without antibiotics (Sambrook and Russell, 2001). Recombinant plasmids were selected by plating bacteria onto LB medium containing ampicillin (100 µg/ml) and X-GAL (50 mg/ml). White colonies containing the recombinant plasmids were selected and grown at 37 ^{o}C overnight in LB liquid medium containing ampicillin (100 µg/ml) for plasmid DNA extraction which was done using the QIAprep Spin Mini Prep kit (Qiagen). Randomly selected white clones were sequenced as described below.

Sequencing and Annotation

Randomly selected white clones were sequenced using the M13 forward primer by the Interdisciplinary Center for Biotechnology Research (ICBR; University of Florida, Gainesville). Sequences obtained were exposed to a 'VecScreen' algorithm (http://www.ncbi.nlm.nih.gov/) to remove vector contamination. Using ClustalW program overlapping sequences were discarded. Genomic sequences obtained were identified by homology searches (Blastn and Blastx) in different databases (non-redundant sequences in National Center for Biotechnology Information

(NCBI), EST sequences in NCBI, and the potato and rice EST database at The Institute for Genomic Research (TIGR)).

Results

A combination of degenerate primers (Bertioli et al., 2003) was used for DNA amplification of RGA sequences from the root-knot nematode resistant cultivar, NemaTAM. The resulting PCR products range in size from 500 bp to 800 bp. A representative gel is shown in Fig. 5-1.

Genomic sequences obtained upon cloning and sequencing of the purified PCR products were identified by homology searches (Blastn and Blastx) in different databases and results are summarized in Table 5-2 and Fig. 5-2. Homologies with the NBS-LRR of *R* genes or with the RGL sequences that had been cloned from other plant species were found. In total, 234 genomic clones were sequenced, and 145 showed significant hits to known sequences in the NCBI database. Homology searches revealed that 26% of the sequences belonged to the section *Arachis* and another 18% belonged to other legumes such as *Medicago truncatula, Vigna mungo* and *Cicer arietinum.* Fourty one percent of the clones have sequences with significant homology to genes found in *Manihot esculanta, Zingiber officinalis, Populus tomentosa, Ipomea batatus, Coffea arabacia, Oryza sativa, Poincirus trifoli, Prunus avium, Triticum aestivum, Solanum demissum, Cicer arietinum,* and *Arabidopsis thaliana*. Of the remaining clones, 15% have sequences related to genes found in microorganisms such as *Erwinia amylovora* and *Campylobacter jejuni* (Fig. 5-2). Interestingly, one NemaTAM genomic clone shared conserved motifs found in the well-characterized tomato root-knot nematode resistance gene, *Mi* (Milligan et al., 1998).

Putative functions of 88% of the NemaTAM sequences were assigned. The remaining 12% of the sequences in this study did not show significant homology to those in public databases.

Sequences were classified into four different major groups according to the putative function of their homologous genes in the databases. The distribution of the genes is illustrated in Fig.5-3. The largest set of genes (70%) was assigned to the disease resistance/response to stress /plant defense group. Within this group, genes that belong to the NBS-LRR class (53%) occupied the largest percentage, followed by TIR (8%), CC-NBS (5%), and TIR-NBS (4%). Catalases (9%) and Reverse Transcriptase (9%) formed the next largest groups. The remaining 12% of sequences were grouped as hypothetical proteins.

Sequence analysis and identification of genomic clone *NRP3D-4*

A clone of approximately 771 bp amplified with P1B-fwd and P3D-rev and designated as *NRP3D-4*, showed high homology to previously identified resistance genes from wild *Arachis* species (Bertioli et al., 2003). Figure 5-4 shows the restriction map of *NRP3D-4*. The genomic clone lacks unique restriction sites for Bam HI, Xho I, Xho II, Eco RI, Sma I and Xba I. The unique restriction sites present are Sac I, Sac II, Pst I, Hind III, Sau3A1, Dra I, Dra III, and Not I.

The partial nucleotide sequence of *NRP3D-4* and its deduced amino acid sequence are shown in Fig. 5-5. The position of the first potential ATG initiation codon in the sequence is found at position 33. The 3' untranslated region contains a sequence that is identical to the consensus polyadenylation site (AATAAA) located at position 651 bp. The first ATG starts an open reading frame at nucleotide 33, and encodes a polypeptide of 193 amino acids with a calculated molecular mass of 20.9 kDa and a pI of 8.27. The hydropathy profile of the protein, deducted by the algorithm of Kyte and Doolittle (1982), indicates that it is largely hydrophilic in nature (Fig. 5-6). Moreover, according to the k-NN algorithm (Horton and Nakai, 1997) used in the PSORT program, the protein was predicted, with a probability of ~50%, to be an intracellular and cytoplasmic in localization. Additional analysis based on consensus sequences for conserved functional domains (PROSITE databases) showed that the NRP3D-4 protein contains an NB-

ARC domain that begins at amino acid 20 (Kinase Ia) and ends at amino acid 200 (HD motif) (Fig. 5-7). This region consists of conserved motifs such as the phosphate-binding P-loop (Kinase Ia motif), with the consensus sequence GxGxxGR(T/S) where x stands for any residue, a hydrophobic HD motif with consensus sequence GLPLxL and two additional kinase domains, kinase 2 with consensus sequence LLVLDDVW/D and kinase 3a with consensus sequence GSRIII/ATTRD (Soriano et al., 2005). Mutation in Kianse Ia domain causes reduced ATP binding indicating possible role of this motif in ATP binding (Tameling et al., 2002). The kinase 2 domain is proposed to coordinate the metal ion binding required for phospho-transfer reactions. Similarly, the arginine in the kinase 3a domain interacts with purine base of ATP in other proteins (Traut, 1994).

Comparisons of the nucleotide sequence and the predicted amino acid sequence against other known sequences were conducted with BLAST and the latest versions of GenBank, EMBL, GenPep and SwissProt databases. Initial analysis of the NRP3D4-deduced amino acid sequence showed significant identity (99% and 98%) with the deduced amino acid sequences of two clones (AY157789 and AY157947) which were isolated from *Arachis cardenasii* and *Arachis stenosperma* encoding two putative resistance proteins (Bertioli et al.,2003) (Fig.5-8). It was also found that the NB-ARC domain of NRP3D-4 has high similarity to the NB-ARC domains of these two proteins (Fig. 5-8). There was also 52% identity at the amino acid level with the root-knot nematode resistance protein Mi-1.1 protein of *Lycopersicon esculentum* (AAC67237).

Expression of the *NRP3D-4* gene in peanut infected by *M. arenaria*

The transcript level of *NRP3D-4* in roots of root-knot nematode resistant and susceptible cultivars was determined after infection with *M. arenaria* race 1 by semi-quantitative RT-PCR analysis. Total RNA was isolated from roots of the root-knot nematode-resistant cultivar

NemaTAM and the susceptible cultivar Florunner at five time points: 0, 12, 24, 48, and 72 h after inoculation/infection with *M. arenaria* race 1. Based on the RT-PCR products, NRP3D-4 was expressed at 0, 12 and 24 hours after inoculation in NemaTAM. NRP3D-4 was not expressed in Florunner roots at any time point tested (Fig.5-9).

Discussion

Knowledge of the cultivated peanut genome is limited and only in recent years have molecular techniques been used to interpret its genome organization. Consequently, the best strategy to identify candidate R genes/ NBS encoding sequences from peanut is to use the information obtained from model plants and public data bases. In the present study, degenerate primers designed by Bertioli et al. (2003) that bind to regions encoding NBS motifs were used to isolate R genes from NemaTAM which is known to be resistant to root-knot nematodes due to introgression of sequences from *A. cardenasii* (Bendezu and Starr, 2003). Among the defense related genes amplified, a majority belonged to the TIR type (12%) and none belonged to the non-TIR type. This finding is in agreement with the previous reports by Bertioli et al., (2003) and Yuksel et al., (2005) that legumes in general contain fewer non-TIR type genes than TIR type genes.

The *R* gene sequences isolated from NemaTAM can be explored further as potential markers to trace resistance genes in peanut breeding programs through marker-assisted selection. For instance, Seah et al. (2001) used *R* gene derived markers to trace integrated rust resistance in wheat. Similarly, R- gene markers have been used to assess genetic diversity and for QTL analysis (Feuillet et al., 1997; Chen et al., 1998).

Homology searches further revealed that 38 (26%) of the genomic clones belonged to *Arachis* species, out of which, 18 (13%) clones showed high homology to *A. cardenasii.* The remaining 20 clones belonged to *Arachis stenosperma* and *Arachis hypogaea*. Among the clones

that showed homology to *A. cardenasii*, one clone *NRP3D-4*, showed significant homology to a well-characterized tomato root-knot nematode resistance gene, *Mi* and two cDNA clones (AY157789 and AY157947) that code for putative R proteins from *A. cardenassi* and *A. stenosperma* (Fig.5-8). Hydropathicity plot analysis indicates that the NRP3D-4 protein is hydrophilic and possibly located in the cytoplasm due to the absence of signal sequences (Fig.5-5). Similarly, Williamson (1998) reported that *Mi* and related NBS-LRR proteins lack signal sequences and are thus likely to be cytoplasmically located.

In NemaTAM roots, *NRP3D-4* is expressed prior to nematode infection and continues to be expressed at 12 and 24 h post infestation. However, at 48 and 72 h after infection there is no detectable expression of *NRP3D-4*. In Florunner roots, *NRP3D- 4* transcripts were not detected any time point. This indicates that *NRP3D-4* may not be present in Florunner or it may be present but not expressed.

Taken together, the above results suggest that *NRP3D*-4 is a major sequence introgressed from *A. cardenassi* into NemaTAM may be responsible for resistance to *M. arenaria*, atleast at the early stages of interaction. However, additional studies are necessary to determine *NRP3D-4* expression at the protein level following root-knot nematode infection and its specific cellular localization within NemaTAM roots. Also, a direct relationship needs to be established between expression of *NRP3D-4* and root-knot nematode resistance either through future forward or reverse genetic analyses.

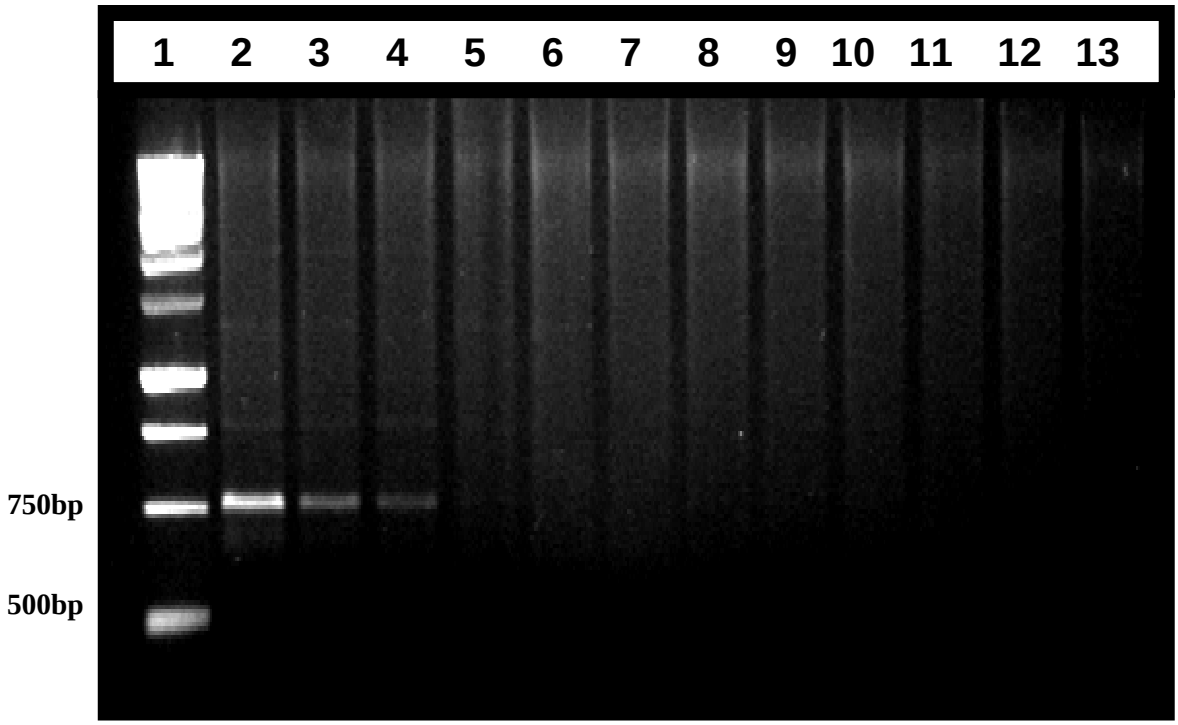

Figure 5-1. PCR products obtained after optimization of degenerate primers P1B-fwd and P3D-rev. Lane 1 = 1 KB DNA Ladder; Lane 2 = Amplified product at annealing temperature 50 ^{o}C; Lane 3 = Amplified product at annealing temperature 50.3 ^{o}C; Lane 4 = Amplified product at annealing temperature 51.4 ^{o}C; Lane 5 – 13 = No amplified products between annealing temperatures 53.2 ^{o}C to 70.5 ^{o}C.

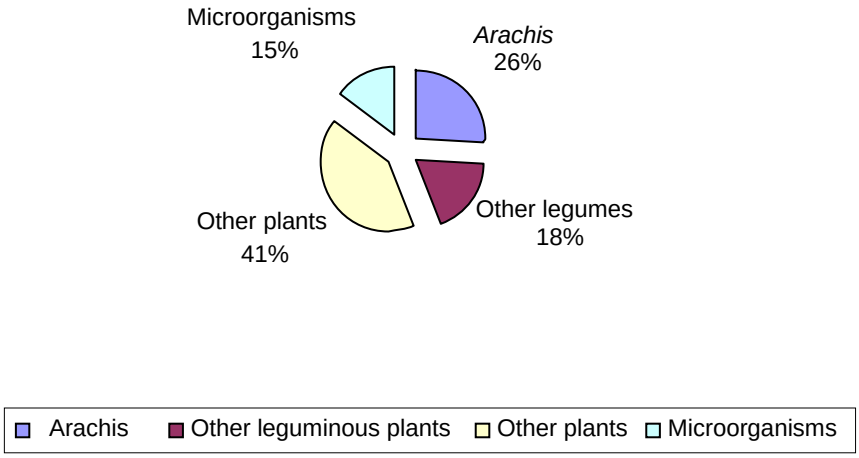

Figure 5-2. Sequence categorization based on homology searches in GenBank.

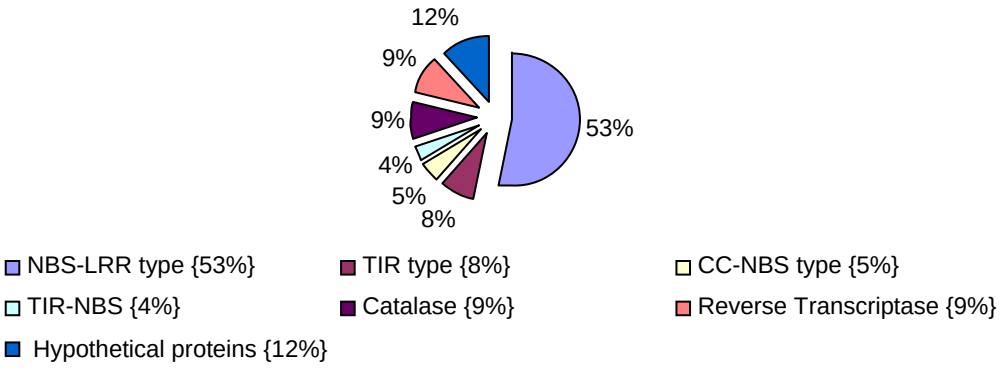

Figure 5-3. Major Functional Categories of NemaTAM. NBS = Nucleotide Biding Site; LRR = Leucine Rich Repeats; TIR = Toll and Interleukin-1 Receptor; CC = Coiled-Coil

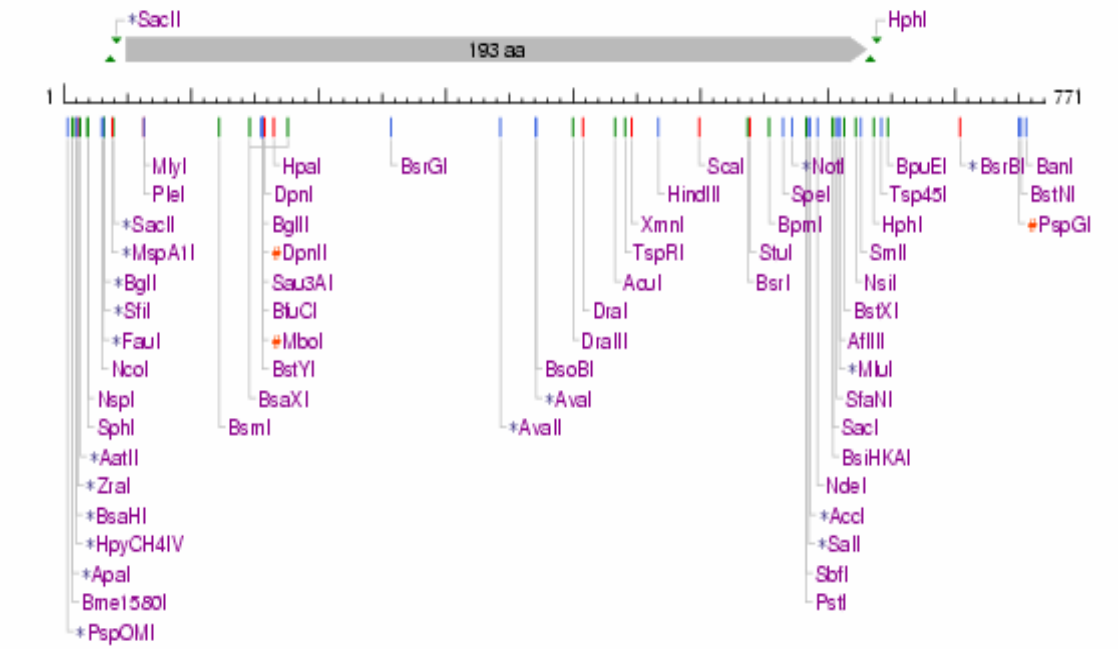

Figure 5-4. Restriction map of peanut (*Arachis hypogaea* L.) genomic clone NRP3D-4.

```
ttgggcccgacgtcgcatgctcccggccgccatggccgcgggattggg**atg**gggggggggg
                                                    **M**  G  G  G
gggaaaacgactcttgcctctgttgtctgtaaaagaatcagaagagaatttgaagattat
 G  K  T  T  L  A  S  V  V  C  K  R  **I  R  R  E  F  E**  D  Y
tgcattctccgagttggagatgtttcaaaggaaggagatctagttaacttacaaaatcaa
 C  I  L  R  V  G  D  V  S  K  E  G  D  L  V  N  L  Q  N  Q
cttctttcacatctgaaactaaggagcagagtaattgaaactttagtccaaggaagggac
 L  L  **S  H  L**  K  L  R  S  R  V  I  E  T  L  V  Q  G  R  D
aacatcagaaaccttctgtacaaaaaaaggattcttattgtcctagatgatgtgagaact
 N  I  R  N  L  L  Y  K  K  R  I  L  I  V  L  D  D  V  R  T
atagaacagctagagaatttggtaggaaacaaggaatggtttggtccgggaagcagaata
 I  E  Q  L  E  N  L  V  G  N  K  E  W  F  G  P  G  S  R  I
gttgttacaactcgggacaagaacctgcttagttcacatggtgcgtttaaaatctatgag
 V  V  T  T  R  D  K  N  L  L  S  S  H  G  A  F  K  I  Y  E
atggaagtcttgaacactgatgaatcccttcagctctttcatcaagaagctttcaaagga
 M  E  V  L  N  T  D  E  S  L  Q  L  F  H  Q  E  A  F  K  G
gagctaccaaaagaagagtacttggagttgtcgaaaaggtttgtgagctatactggaggc
 E  L  P  K  E  E  Y  L  E  L  S  K  R  F  V  S  Y  T  G  G
ctccccttgacccccaacctaatcactagtgcggccgcctgcaggtcgaccatatgggag
 L  P  L  T  P  N  L  I  T  S  A  A  A  C  R  S  T  I  W  E
agctcccaacgcgttggatgcatagct**tga**gtattctatagtgtcacc**taaataa**cttgg
 S  S  Q  R  V  G  C  I  A  -
cgtaatcatggtcatagctgtttcctgtgtgaaattgttatccgctcacaattccacaca

acatacgagccggaagca**taa**agtgtaaagcctggggtgcctaatgagtg
```

Figure 5-5. Nucleotide and deduced amino acid sequences of the genomic clone *NRP3D-4*.From top to bottom, the (atg) initiation codon, (IRREFE) mitochondrial targeting sequence, (SKL) signal for peroxisomal protein, the first stop codon (tga), and the putative polyadenylation signal (taaataa) are in bold. Two additional stop codons are underlined. The deduced amino acid sequence is below the nucleotide sequence.

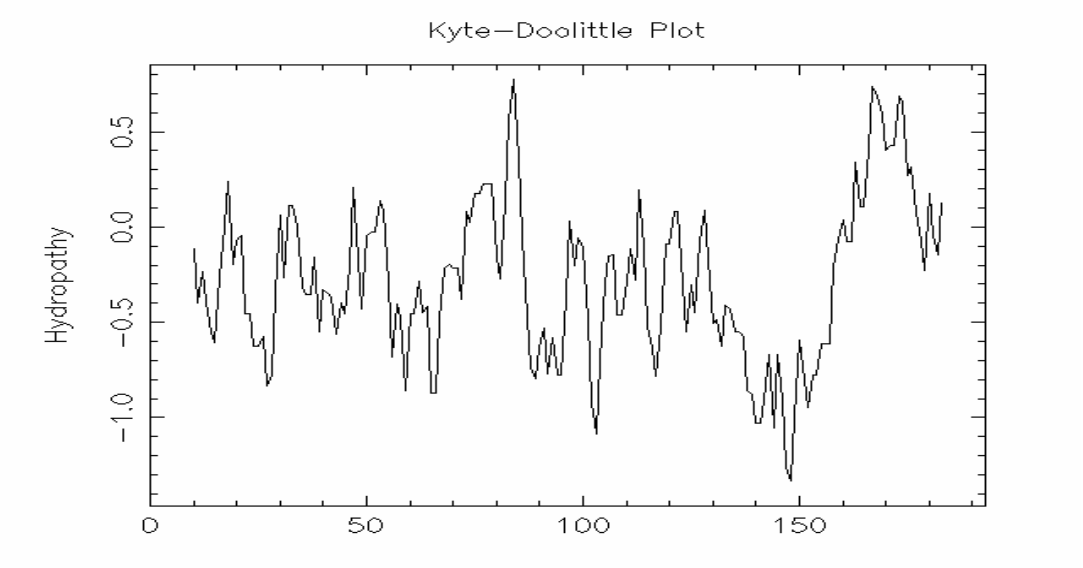

Figure 5-6. Hydropathy profile of the protein deduced from NRP3D-4.

Figure 5-7. Graphical overview of peanut NRP3D-4 representing location of the conserved NB-ARC domain. This domain N-terminal motif consists of 181 residues (20 through 200; E value 1e-20). Numbers indicate amino acid residue.

```
                                          Kinase 1a
NRP3D-4           --------------LGPTSHAPGRHGRGIGGGGGKTTLASVVCKRIRREFEDYCILRVGD 46
A.cardenasii      ------------------------------------LASVVCKRIRREFEDYCILRVGD 27
A.stenosperma     ------------------------------------LASVVCKRIRREFEDYCILRVGD 27
Lycopersicon      TVGFEEETNLILRKLTSGSADLDVISITGMPGSGKTTLAYKVYN--DKSVSSRFDLRAWC 58
                                             ******  * :   :....   **.

                                                        Kinase 2
NRP3D-4           VSKEGDLVNLQNQLLSHLKLRSRVIETLVQGRDNIRNLLYKKRILIVLDDVRTIEQLENL 106
A.cardenasii      VSKEGDLVNLQNQLLSHLKLRSRVIETLVQGRDNIRNLLYKKRILIVLDDVRTIEQLENL 87
A.stenosperma     VSKEGDLVNLQNQLLSHLKLRSRVIETLVQGRDNIRNLLYKKRILIVLDDVRTIEQLENL 87
Lycopersicon      TVQGCDEKKLLNTIFSQVSDSDSKLSENIDVADKLRKQLFGKRYLIVLDDVWDTTTWDEL 118
                  . :  *  :* * ::*::.  .  :.  ::  *::*: *: ** *******      ::*
                        Kinase 3a
NRP3D-4           VGNKEWFGPGSRIVVTTRDK-NLLSSHGAFKIYE-EVLNTDESLQLFHQEAFKGELPKEE 164
A.cardenasii      VGNKEWFGPGSRIVVTTRDK-NLLSSHGVFKIYEMEVLNTDESLQLFHQEAFKGELPKEE 146
A.stenosperma     VGNKEWFGPGSRIVVTTRDK-NLLSSHGVFKIYEMEVLNTDESLQLFHQEAFKGELPKEE 146
Lycopersicon      TRPFPESKKGSRIILTTREKEVALHGKLNTDPLDLRLLRPDESWELLEKRAFGNESCPDE 178
                  .        ****::***:*   * .:   .  : .:*..*** :*:.:.** .*   :*
                        HD motif
NRP3D-4           YLELSKRFVSYTGGLPLTPNLITSAAACRSTIWESSQRVG----------CIAVFYSVTI 214
A.cardenasii      YLELSKRFVSYTGGLPL------------------------------------------- 163
A.stenosperma     YLELSKRFVSYTGGLPL------------------------------------------- 163
Lycopersicon      LLDVGKEIAENCKGLPLVADLIAGVIAGREKKRSVWLEVQSSLSSFILNSEVEVMKVIEL 238
                   *::.*.:..   ****

NRP3D-4           AWRNHGHSCFLCEIVIRSQFHTTYEPEASVKPGVPNE--- 251
A.cardenasii      ---------------------------------------
A.stenosperma     ---------------------------------------
Lycopersicon      SYDHLPHHLKPCLLYFASFPKDTSLTIYELNVYFGAEGFV 278
```

Figure 5-8. Comparisons of the deduced amino acid sequence of the NemaTAM protein NRP3D-4 with sequences from *A. cardenasii* (AY157789), *A. stenosperma* (AY157947), and *Lycopersicon* Mi-1.1 root-knot nematode resistance protein (AAC67237).

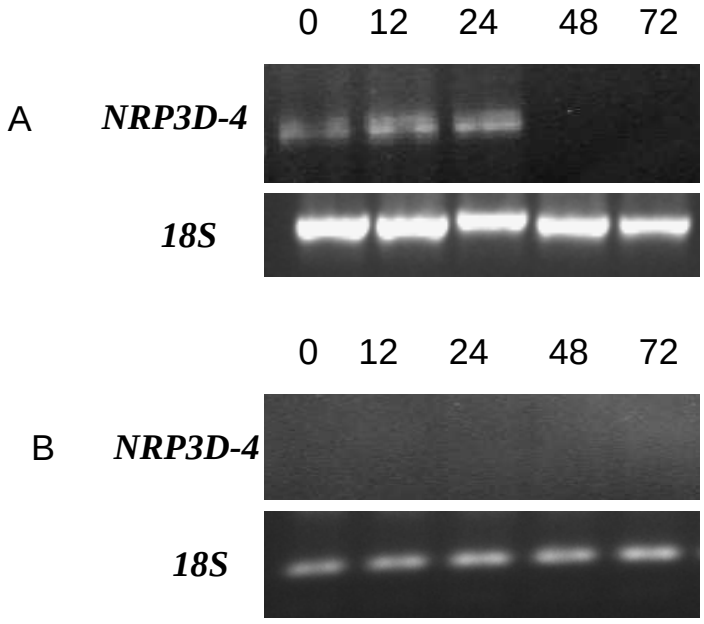

Figure 5-9. RT-PCR analysis of the expression of NRP3D-4 in NemaTAM and Florunner. A) Expression pattern of NRP3D-4 in roots of NemaTAM 0, 12, 24, 48, 72 h after infestation with *M. arenaria*. B) Expression pattern of NRP3D-4 in roots of Florunner 0, 12, 24, 48, 72 h after infestation with *M. arenaria*.

Table 5-1. Primer sequences used to amplify RGAs in NemaTAM (Bertioli et al., 2003).

Primer	Motif	Motif sequence	Primer sequence[a]
P1a-fwd	P-loop	GM[PG]G[IVS]GKTT	GGIATGCCIGGIIIIGGIAARACIAC
P1b-fwd	P-loop	GM[PG]G[IVS]GKTT	GGIATGGGIGGIIIIGGIAARACIAC
P3a-rev	GLPL	GLPL[TAV][LAV][KND]	AIITYIRIIYIAGIGGYAAICC
P3d-rev	GLPL	GLPL[TAV][LAV][KND]	AIITYIRIIRYYAAIGGIAGICC
LM638	P-loop	GGVGKTT	GGIGGIGTIGGIAAIACIAC
RNBS-D-rev	RNBS-D	CFLYCALFP	GGRAAIARISHRCARTAIVIRAARC

[a]I, inosine. Codes for degenerate positions are: R, A/G; Y, C/T; S, G/C; H, A/C; V, A/C

Table. 5-2. Identification of genomic clones from the root-knot nematode resistant cultivar NemaTAM.

Clone #	Accession # of genomic clone	Accession # of matching sequence	Best E-value (BLASTX)	Source of matching sequences	Matching sequence from database
			Resistant Proteins		
NRGH3B12	Ne36037	AAN85395.1	7E-75	*Arachis cardenasii*	Resistance Proteins (NB-ARC domain)
NRGH3H11	Ne36038	AAN85041	6E-36	*Arachis cardenasii*	Resistance Proteins (NB-ARC domain)
NRGH3H10	Ne363039	AAN85377	3E-09	*Arachis cardenasii*	Resistance Proteins (NB-ARC domain)
NRGH3H09	Ne363040	AAN85401	6E-17	*Arachis cardenasii*	Resistance Proteins (NB-ARC domain)
NRGH3H03	Ne363045	AAN5401	2E-33	*Arachis cardenasii*	Resistance Protein
NRGH3H02	Ne363046	AAO20377	3E-55	*Arachis hypogaea*	Resistance Protein (NBS LRR type)
NRGH3H01	Ne363047	AAX81299	3E-52	*Arachis hypogaea*	Resistance Protein pLTR (NBS LRR type)
NRGH3G11	Ne363049	AAX71066	7E-81	*Arachis hypogaea*	Resistance Protein PRG (NBS-LRR)
NRGH3G10	Ne363050	AAO38219	3E-36	*Manihot esculanta*	Resistance Protein (NBS LRR type)(RCa8)
NRGH3G09	Ne363051	AAO20357	2E-76	*Arachis stenosperma*	Resistance Protein (TIR-NBS)
NRGH3G08	Ne363052	AAO20357	3E-72	*Arachis stenosperma*	Resistance Protein (TIR-NBS)
NRGH3G05	Ne363055	AAO20357	4E-81	*Arachis stenosperma*	Resistance Protein
NRGH3G01	Ne363058	AAX71086	7E-73	*Arachis hypogaea*	Resistance Protein
NRGH3F10	Ne363061	AAO20357	6E-78	*Arachis stenosperma*	Resistance Protein
NRGH3F06	Ne363063	AAO20357	3E-81	*Arachis stenosperma*	Resistance Protein PLTR
NRGH3F03	Ne363066	AAX81299	6E-78	*Arachis hypogaea*	Resistance Protein PLTR
NRGH3F01	Ne363068	AAO20357	5E-46	*Arachis stenosperma*	Resistance Protein (NBS-LRR)
NRGH3E12	Ne363069	AAO20357	5E-74	*Arachis stenosperma*	Resistance Protein (TIR-NBS)
NRGH3E10	Ne363071	AAN85380	6E-65	*Arachis cardenasii*	Resistance Protein
NRGH3E08	Ne363073	AAV70155	3E-60	*Arachis cardenasii*	Resistance Protein like Protein
NRGH3E07	Ne363074	AAO38219	3E-40	*Manihot esculanta*	Resistance Protein RCG8 (NBS-LRR)
NRGH3E06	Ne363075	CAN63338	7E-28	*Glycine max*	NBS-LRR type disease resistance protein
NRGH3E02	Ne363079	AAO38219	5E-35	*Manihot esculanta*	Disease Resistance Protein RCA8
NRGH3D12	Ne363081	AA85395	4E-85	*Arachis cardenasii*	Resistance Protein
NRGH3D07	Ne363085	ABE86887	6E-42	*Medicago truncatula*	Disease Resistance Protein
NRGH3D06	Ne363086	AAO38219	8E-35	*Manihot esculanta*	Resistance Protein RCA8
NRGH3C11	Ne363092	AAO38219	2E-55	*Manihot esculanta*	Resistance Protein RCA8

Table 5-2(Continued)

Clone #	Accession # of genomic clone	Accession # of matching sequence	Best E-value (BLASTX)	Source of matching sequences	Matching sequence from database
NRGH3C10	Ne363093	AAY81228	7E-32	*Arachis hypogaea*	Resistant Putative PLTR
NRGH3C09	Ne363094	AAN85395	3E-89	*Arachis cardenasii*	Resistance Protein
NRGH3C08	Ne363095	AAN85395	5E-64	*Arachis cardenasii*	Resistance Protein
NRGH3C07	Ne363096	AAO38219	8E-40	*Manihot esculanta*	Resistance Protein RCA8
NRGH3C06	Ne363097	AAO38220	2E-40	*Manihot esculanta*	Resistance Protein RCA8
NRGH3C03	Ne363100	AAN85395	8E-80	*Arachis cardenasii*	Resistant Protein
NRGH3C02	Ne363101	AAV70115	1E-52	*Arachis cardenasii*	Resistant Protein like Protein
NRGH3C01	Ne363102	AAN85395	6E-76	*Arachis cardenasii*	Resistant Protein like Protein
NRGH3H12	Ne363103	AAN85401	8E-35	*Arachis cardenasii*	Resistant Protein like Protein
NRGH3B08	Ne363107	ABE84400	8E-48	*Medicago truncatula*	Disease Resistance Protein
NRGH3B07	Ne363108	AAO38219	3E-40	*Arachis esculanta*	Resistant Protein
NRGH3B06	Ne363109	AAN85380	2E-69	*Arachis cardenasii*	Resistant Protein
NRGH3B01	Ne363112	AAX71046	1E-29	*Arachis hypogaea*	Resistant Protein
NRGH3A12	Ne363113	AAN85395	4E-49	*Arachis cardenasii*	Resistant Protein
NRGH3A11	Ne363114	AAV70115	9E-68	*Arachis cardenasii*	Resistant Protein like Protein
NRGH3A08	Ne363116	AAO38219	4E-39	*Manihot esculanta*	Resistant Protein RCA8
NRGH3A06	Ne363118	AAO20349	9E-26	*Arachis stenosperma*	Resistant Protein
NRGH3A04	Ne363120	AAO38219	2E-14	*Manihot esculanta*	Resistant Protein RCA8
NRGH3A02	Ne363122	AAN85380	5E-55	*Arachis cardenasii*	Resistant Protein
NRGH2A06	Ne262150	QO2940	1E-08	*Burkholderia ceparia*	Beta lactamase precursor
NRGH2A09	Ne262153	ABD33309	1E-08	*Populus tomentosa*	NBS-LRR type disease resistance protein
NRGH2B04	Ne262160	ABO21765	7E-14	*Vigna mungo*	Disease resistance protein (NBS-LRC)
NRGH2B07	Ne262163	ABN42888	7E-32	*Phaseolus vulgaris*	Resistance Protein
NRGH2C01	Ne262169	AAO23077	1E-33	*Glycine max*	Resistance Protein (NB-ARC) (LRR Protein)
NRGH2C03	Ne262171	CAC86491	5E-11	*Cicer arietinum*	Resistance Protein RGA-B
NRGH2C07	Ne262175	ABD28507	4E-25	*Medicago truncatula*	LRR type resistance protein

Table 5-2(Continued)

Clone #	Accession # of genomic clone	Accession # of matching sequence	Best E-value (BLASTX)	Source of matching sequences	Matching sequence from database
NRGH2C09	Ne262177	ABN42879	1E-41	*Phaseolus vulgaris*	Resistance Protein (NB-ARC)
NRGH2C10	Ne262178	ABC59491	8E-34	*Populus tomentosa*	NBS-LRR type disease resistance protein
NRGH2C11	Ne262179	AA023077	2E-28	*Glycine max*	Resistance Protein (NB-ARC)
NRGH2D01	Ne262181	AAZ07904	5E-15	*Ipomea batatus*	NBS-LRR type resistance protein
NRGH2D02	Ne262182	ABC59503	7E-37	*Populus tomentosa*	NBS-LRR type resistance protein
NRGH2D06	Ne262186	ABA54554	3E-04	*Triticum aestivum*	NBS-LRR type resistance protein
NRGH2D07	Ne262187	ABC59503	6E-39	*Populus tomentosa*	NBS-LRR type resistance protein
NRGH2D09	Ne262189	ABB00419	6E-17	*Capsicum annum*	NB-ARC resistance protein
NRGH2E02	Ne262194	AAT39962	3E-16	*Solanum demissum*	late blight resistance protein
NRGH2E03	Ne262195	ABC59502	2E-38	*Populus tomentosa*	NBS-LRR disease resistance protein
NRGH2E02	Ne262196	ABC59503	3E-11	*Populus tomentosa*	NBS-LRR disease resistance protein
NRGH2E05	Ne262197	AAZ07904	2E-26	*Ipomea batatus*	NBS-LRR disease resistance protein
NRGH2E07	Ne262199	AAO95247	7E-19	*Solanum tuberosum*	Resistance Protein (NB-ARC)
NRGH2E08	Ne262200	CAC82607	2E-07	*Coffea arabacia*	NBS-LRR type resistance protein
NRGH2E09	Ne262201	ABN42889	4E-46	*Phaseolus vulgaris*	NBS-LRR type resistance protein
NRGH2E12	Ne262204	ABC59503	6E-39	*Populus tomentosa*	NBS-LRR type disease resistance protein
NRGH2F01	Ne262205	AAT39962	1E-06	*Solanum demissum*	late blight resistance protein
NRGH2F02	Ne262206	AAZ07904	6E-16	*Ipomea batatus*	NBS-LRR type resistance protein
NRGH2F03	Ne262207	IT48898	4E-05	*Arabidopsis thaliana*	disease resistance protein RPP8 (NB-ARC)
NRGH2F06	Ne262210	ABN42889	1E-36	*Phaseolus vulgaris*	NBS-LRR disease resistance protein
NRGH2F08	Ne262212	ABC59503	4E-13	*Populus tomentosa*	NBS-LRR disease resistance protein
NRGH2F09	Ne262213	CAC82598	3E-21	*Coffea arabacia*	NB-ARC resistance protein
NRGH2F10	Ne262214	ABC59503	1E-30	*Populus tomentosa*	NBS-LRR type resistance protein
NRGH2F11	Ne262215	ABC59503	7E-32	*Populus tomentosa*	NBS-LRR type resistance protein
NRGH2F12	Ne262216	AAZ07904	5E-18	*Ipomea batatus*	NBS-LRR type resistance protein
NRGH2G01	Ne262217	AAT39962	2E-15	*Solanum demissum*	late blight resistance protein
NRGH2G02	Ne262218	AAT39962	7E-16	*Solanum demissum*	late blight resistance protein
NRGH2G05	Ne262221	AAZ07910	5E-07	*Ipomea batatus*	NBS-LRR type disease resistance protein
NRGH2G06	Ne262222	AAZ07910	2E-18	*Ipomea batatus*	NBS-LRR type disease resistance protein

Table 5-2(Continued)

Clone #	Accession # of genomic clone	Accession # of matching sequence	Best E-value (BLASTX)	Source of matching sequences	Matching sequence from database
NRGH2G10	Ne262226	AAT69649	1E-27	*Oryza sativa*	NBS-LRR type resistance protein
NRGH2G11	Ne262227	AAU89649	2E-15	*Poncirus trifoli*	NB-ARC type resistance protein
NRGH2G12	Ne262228	ABN42889	1E-47	*Phaseolus vulgaris*	NB-ARC type resistance protein
NRGH2H01	Ne26229	AAZ99766	5E-13	*Triticum aestivum*	NBS- LRR type RGA (resistant protein)
NRGH2H09	Ne262237	AAU89649	8E-15	*Poncirus trifoli*	NB-ARC type resistance protein
NRGH2H11	Ne262239	ABC59503	7E-29	*Populus tomentosa*	NBS-LRR type resistance protein
NRGH2H12	Ne262240	AAZ07904	4E-19	*Ipomea batatus*	NBS-LRR type resistance protein
NRGH1B02	Ne250949	ABH06479	4E-11	*Prunus avium*	NBS-LRR type resistance protein
NRGH1B04	Ne250951	AB148864	1E-80	*Arachis hypogaea*	NBS-LRR type resistance protein
NRGH1C07	Ne250965	ABN42879	2E-06	*Phaseolus vulgaris*	NB-ARC type resistance protein
NRGH1C10	Ne250968	ABN42879	7E-05	*Phaseolus vulgaris*	NB-ARC type resistance protein
NRGH1E07	Ne250980	ABI18151	4E-57	*Arachis hypogaea*	NBS-LRR type resistant protein GnRGC5
TIR type Resistance Protein					
NRGH2A12	Ne262156	ABD28703	2E-29	*Medicago truncatula*	TIR type resistance protein
NRGH2B08	Ne262164	ABD28703	1E-30	*Medicago truncatula*	TIR type resistance protein
NRGH2C05	Ne262173	ABD28703	2E-28	*Medicago truncatula*	TIR type resistance protein
NRGH2C06	Ne262174	ABD28703	2E-36	*Medicago truncatula*	TIR type resistance protein
NRGH1B06	Ne250953	ABD28703	1E-24	*Medicago truncatula*	TIR type resistance protein
NRGH1B07	Ne250954	ABD28703	3E-37	*Medicago truncatula*	TIR type resistance protein
NRGH1C04	Ne250962	ABD28703	4E-13	*Medicago truncatula*	TIR type resistance protein
NRGH1C08	Ne250966	ABD28703	8E-41	*Medicago truncatula*	TIR type resistance protein
NRGH1E10	NE250983	ABD28703	3E-32	*Medicago truncatula*	TIR type resistance protein
Beta Lactamase Precursor					
NRGH2A06	Ne262150	QO2940	1E-08	*Burkholderia ceparia*	Beta lactamase precursor
Catalase					
NRGH3H06	Ne363043	Q59296	3E-09	*Campylobactor jejuni*	Catalase
NRGH3H05	Ne363044	Q59296	2E-09	*Campylobactor jejuni*	Catalase
NRGH3G07	Ne363053	XP_731877	2E-09	*Campylobactor jejuni*	Catalase

Table 5-2(Continued)

Clone #	Accession # of genomic clone	Accession # of matching sequence	Best E-value (BLASTX)	Source of matching sequences	Matching sequence from database
NRGH3G04	Ne363056	ABK60177	2E-09	*Campylobactor jejuni*	Catalase
NRGH3G02	Ne363057	ABK60177	2E-09	*Campylobactor jejuni*	Catalase
NRGH3C12	Ne363091	Q59296	2E-09	*Campylobactor jejuni*	Catalase
NRGH3B11	Ne363104	Q59296	2E-09	*Campylobactor jejuni*	Catalase
NRGH3B09	Ne363106	Q59296	2E-09	*Campylobactor jejuni*	Catalase
				Hypothetical Protein	
NRGH2B12	Ne262168	AAA72620	1E-08	Synthetic Protein	Diptheria Toxin Protein DT-201
NRGH2B03	Ne262159	AAA72620	1E-08	Synthetic Construct	Produces Diphtheria toxin
NRGH3G07	Ne363053	XP_731877	3E-12	*Cicer arietinum*	Reverse Transcriptase
NRGH3G04	Ne363056	ABK60177	1E-16	*Zingiber officinalis*	Putative Reverse Transcriptase
NRGH3F07	Ne363062	ABK60177	1E-16	*Zingiber officinalis*	Putative Reverse Transcriptase
NRGH3E11	Ne363070	ABK60177	1E-16	*Zingiber officinalis*	Putative Reverse Transcriptase
NRGH3E09	Ne363072	ABK60177	8E-17	*Zingiber officinalis*	Putative Reverse Transcriptase
NRGH3E05	Ne363076	ABK60177	1E-14	*Zingiber officinalis*	Putative Reverse Transcriptase
NRGH3D10	Ne363083	ABK60177	1E-16	*Zingiber officinalis*	Putative Reverse Transcriptase
NRGH3D05	Ne363087	ABK60177	1E-16	*Zingiber officinalis*	Putative Reverse Transcriptase
NRGH3C12	Ne363091	CAD59768	8E-08	*Cicer arietinum*	Putative Reverse Transcriptase
NRGH3C05	Ne363098	ABK60177	1E-16	*Zingiber officinalis*	Putative Reverse Transcriptase
NRGH3B03	Ne363110	ABK60177	1E-16	*Zingiber officinalis*	Putative Reverse Transcriptase
NRGH2A03	Ne262147	CAL37000	3E-09	*Platanus acerifolia*	Putative Reverse Transcriptase
NRGH2B05	Ne262161	CAL37000	3E-09	*Platanus acerifolia*	Putative Reverse Transcriptase
NRGH1E08	Ne250981	CAL37000	3E-09	*Platanus acerifolia*	Reverse transcriptase
				Ribonuclease H	
NRGH2A08	Ne262152	NP758764	2E-12	*Erwinia amylovora*	Ribonuclease H
NRGH2A09	Ne262153	ABD33309	1E-08	*Medicago truncatula*	Ribonuclease H
NRGH1B05	Ne250952	ABI33309	8E-04	*Medicago truncatula*	Ribonuclease H

APPENDIX A
EST CLONES FROM THE ROOT-KNOT NEMATODE RESISTANT CULTIVAR NEMATAM WITH A CUT OFF E-VALUE BELOW 1E-04

Table A-1. EST clones from the root-knot nematode resistant cultivar NemaTAM with a cut off E-value below 1E-04

Clone ID	Accession # of EST clone	Accession # of matching sequence	E-value	Source of matching sequence	Function
SSHPL1F07	Ne469400	XP_754657	2.7	*Aspergillus fumigatus*	RNA recognition motif
SSHPL1A08	Ne469363	BAC14473	0.97	*Oceanobacillus iheyensis*	Late competence protein
SSHPL1H01	Ne469382	EAL30259	0.73	*Drosophila pseudoobscura*	Unknown protein
SSHPL2A12	Ne472915	ABE93082	1.3	*Medicago truncatula*	Unknown protein
SSHPL2C12	Ne472970	BAA00448	1.5	*Mus musculus*	Unknown protein
SSHPL2A10	Ne472917	BAD13492	5	*Chlamydomonas reinhardtii*	Unknown protein
SSHPLA06	Ne469365	AAT71913	2.2	Rock bream iridovirus	Unknown protein
SSHPLA09	Ne469362	XP_754657	0.25	*Aspergillus fumigatus*	RNA recognition motif
SSHPL1H09	Ne469374	ABA86508	8.1	*Drosophila melanogaster*	Ribonuclease inhibitor
SSHPL1A11	Ne469360	AAH54715	0.57	*Danio rerio*	Hypothetical protein
SSHPL1H12	Ne469371	XP_505388	0.74	*Yarrowia lipolytica*	Hypothetical protein
SSHPL1G03	Ne469392	EAM73150	3.6	*Kineococcus radiotolerans*	Hypothetical protein
SSHPL1E06	Ne469413	XP_505388	0.14	*Yarrowia lipolytica*	Hypothetical protein
SSHPL1E09	Ne469410	ABE88542	42.4	*Medicago truncatula*	Hypothetical protein
SSHPL2A06	NE472921	XP_534180	0.77	*Canis familiaris*	Hypothetical protein
SSHPL2A05	Ne472922	XP_640433	8.5	*Dictyostelium discoideum*	Hypothetical protein
SSHPL1H04	Ne469379	EAM73150	1.3	*Kineococcus radiotolerans*	Hypothetical protein
SSHPL1E08	Ne469411	AAW44411	0.32	*Cryptococcus neoformans*	Hypothetical protein
SSHPL1E12	Ne469407	XP_505388	0.32	*Yarrowia lipolytica*	Hypothetical protein
SSHPLE11	Ne469408	XP_754657	1.6	*Aspergillus fumigatus*	RNA recognition motif
SSHPL1D06	Ne469425	XP_700570	1.2	*Danio rerio*	Recombination signal recognition protein
SSHPL1D03	Ne469428	ZP_00787826	2.7	*Streptococcus agalactiae*	Restriction enzyme
SSHPL1D12	Ne469419	NP_573462	2	*Mus musculus*	Ion transport protein
SSHL1C09	Ne469434	XP_744235	2	*Plasmodium chabaudi*	Hypothetical protein

Table A-1(Continued)

Clone ID	Accession # of EST clone	Accession # of matching sequence	E-value	Sourceof matching sequence	Function
SSHPL1C06	Ne469437	CAJ06381	1.2	*Leishmania major*	Hypothetical protein
SSHPL1B03	Ne469452	XP_711078	0.59	*Cadida albicans*	Hypothetical protein
SSHPL2D05	Ne472965	ZP_01122517	0.042	*Robiginitalea biformata*	Hypothetical protein
SSHPL2B11	Ne472983	AAH95705	9.3	*Danio rerio*	Hypothetical protein
SSHPL2C09	Ne472973	XP_637648	3.2	*Dictyostelium discoideum*	Homeodomain (Hox) protein
SSHPL2B03	Ne472990	ABB81899	5.4	*Sclerophasma paresisensis*	NADH dehydrogenase
SSHPL2C08	Ne472974	ZP_01050141	5.5	*Cellulophaga* species	DNA polymerase
SSHPL2C07	Ne472975	XP_001066085	5.5	*Rattus norvegicus*	G-protein receptor
SSHPL2C06	Ne472976	AAV3683838	5.5	*Drosophila melanogaster*	Unknown protein

APPENDIX B
EST CLONES FROM THE ROOT-KNOT NEMATODE SUSCEPTIBLE CULTIVAR FLORUNNER WITH A CUT OFF E-VALUE BELOW 1E-04

Table B-1. EST clones from the root-knot nematode susceptible cultivar Florunner with a cut off E-value below 1E-04

Clone ID	Accession # of EST clone	Accession # of matching sequence	E-value	Source of matching sequence	Function
SSHPL3A06	FL468991	ABE83065	0.039	*Medicago truncatula*	DNA-binding protein
SSHPL2E02	FL472956	EAS32068	6.5	*Coccidioides immitis*	Hypothetical protein
SSHPL3E09	FL469036	AAC32828	0.025	*Glycine max*	Ammonium transporter
SSHPL3E11	FL469034	BAD94515	1	*Oncorhynchus keta*	Peroxisome receptor
SSHPL3E06	FL469039	NP_001035725	5.1	*Danio rerio*	Hypothetical protein
SSHPL3B09	FL469072	BAC42411	0.025	*Arabidopsis thaliana*	Unknown protein
SSHPL2F11	FL472935	EAS32068	3.8	*Coccidioides immitis*	Hypothetical protein
SSHPL2E01	FL472957	AAB39638	0.001	*Glycine max*	Zinc finger protein

LIST OF REFERENCES

Abdel-Momen, S.M., C.E. Simpson, and J.L. Starr. 1998. Resistance of interspecific *Arachis* breeding lines to *Meloidogyne javanica* and an undescribed *Meloidogyne* species. J. Nematol. 30:341-346.

Ahmed, E.M., and C.T. Young. 1982. In: Peanut Science and Technology; H. Pattee and C.T. Young (Eds) Yoakum Texas: Am. Peanut Res. Edu. Soc., 6:35-88.

Andersen, P.C., and D.W. Gorbet. 2002. Influence of year and planting date on fatty acid chemistry of high oleic acid and normal peanut genotypes. J. Agric. Food Chem. 50:1298-1305.

Anderson, P.A., G.J. Lawrence, B.C. Morrish, M.A. Ayliffe, E.J. Finnegan, and J.G. Ellis. 1997. Inactivation of the flax rust resistance gene *M* associated with loss of a repeated unit within the leucine-rich repeat coding region. Plant Cell 9:641-651.

Apel, K., and H. Hirt. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55:373-399.

Atkinson, H.J., P.E. Urwin, and M.J. McPherson. 2003. Engineering plants for nematode resistance. Annu. Rev. Phytopathol. 41:615-39.

Bogdanove, A.J. 2002. Pto update: recent progress on an ancient plant defense signaling pathway. Molecular Plant Pathol. 3:283-288.

Baker, B., P. Zambryski, B. Staskawicz, and S.P. Dinesh-Kumar. 1997. Signaling in plant–microbe interactions. Science 276:726-733.

Baltensperger, D.D., G.M. Prine, and R.A. Dunn. 1986. Root-knot nematode resistance in *Arachis glabrata*. Peanut Sci. 13:78-80.

Barr, A.R., K.J. Chalmers, A. Karakousis, J.M. Kretschmer, S. Manning, R.C.M. Lance, J. Lewis, S.P. Jeffries, and P. Langridge. 1998. RFLP mapping of a new cereal cyst nematode resistance locus in barley. Plant Breed. 117:185-186.

Bendahmane, A., K. Kanyuka, and D.C Baulcombe. 1999 The *Rx* gene from potato controls separate virus resistance and cell death responses. Plant Cell 11:781-791.

Bendezu, I.F., and J.L. Starr. 2003. Mechanism of resistance to *Meloidogyne arenaria* in the peanut cultivar COAN. J. Nematol. 35:115-118.

Bent, A. 1996. Plant disease resistance genes: Function meets structure. Plant Cell 8:1757-1771.

Bent, A.F., B.N. Kunkel, D. Dahlbeck, K.L. Brown, R. Schmidt, J. Giradaut, J. Leung, and B.J. Staskawicz. 1994. RPS2 of *Arabidopsis thaliana*: a leucine-rich repeat class of plant disease resistance genes. Science 265:1856-1860.

Bertioli, D.J., S.C.M. Leal-Bertioli, M.B. Lion, V.L. Santos, G. Pappas, S.B. Cannon, and P.M. Guimaraes. 2003. A large scale analysis of resistance gene homologues in *Arachis*. Mol. Genet. Gen. 270:34–45.

Beyer, K., E. Morrow, X-Min Li, L. Bardina, G.A. Bannon, A.W. Burks, and H.A. Sampson. 2001. Effects of cooking methods on peanut allergenicity. J. Allergy Clin. Immunol. 107:1077-1081.

Bhagwat, A., T.G. Krishna, and C.R. Bhatia. 1997. RAPD analysis of induced mutants of groundnut (*Arachis hypogaea* L.). J. Genet. 76:201-208.

Biezen, E.A.V.D., C.T. Freddie, K. Kahn, J.E. Parker, and J.D.G. Jones. 2002. *Arabidopsis* RPP4 is a member of the RPP5 multigene family of TIR-NB-LRR genes and confers downy mildew resistance through multiple signaling components. Plant J. 29:439-451.

Bird, A.F. 1986. The influence of the actinomycete, *Pasteuria penetrans,* on the host-parasite relationship of the plant-parasitic nematode, *Meloidogyne javanica.* Parasitology 93:571-580.

Bird, A.F., and P.G. Brisbane. 1988. The influence of *Pasteuria penetrans* in field soils on the reproduction of root-knot nematodes. J. Nematol.11:75-81.

Braddock, J.C., C.A. Sims, and S.K. O'Keefe. 1995. Flavor and oxidative stability of roasted high oleic acid peanuts. J. Food Sci. 60:489-493.

Burrows, R.P., A.D.P. Barker, C.A. Newell, and W.D.O. Hamilton. 1998. Plant-derived enzyme inhibitors and lectins for resistance against plant-parasitic nematodes in transgenic crops. Pestic. Sci. 52:176-183.

Burow, M.D., C.E. Simpsom, A.H. Paterson, and J.L. Starr. 1996. Identification of peanut (*Arachis hypogaea* L.) RAPD markers diagnostic of root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood) resistance. Mol. Breed. 2:369-379.

Burow, M.D., C.E. Simpson, J.L. Starr, and A.H. Paterson. 2001. Transmission genetics of chromatin from a synthetic amphiploid in cultivated peanut (*A. hypogaea* L.): Broadening the gene pool of a monophyletic polyploid species. Genetics 159:823-837.

Cai, D.G., M. Kleine, S. Kifle, H.J. Harloff, and N.N. Sandal. 1997. Positional cloning of a gene for nematode resistance in sugar beet. Science 27:832-834.

Cetintas, R., and D.W. Dickson. 2004. Persistence and suppressiveness of *Pasteuria penetrans* to *Meloidogyne arenaria* race 1. J. Nematol. 36:540–549.

Cetintas, R., and D.W. Dickson. 2005. Distribution and downward movement of *Pasteuria penetrans* in field soil. J. Nematol. 37:155–160.

Channer, A.G., and S.R. Gowen. 1988. Preliminary studies on the potential of *Pasteuria penetrans* to control *Meloidogyne* species. Proceedings of brighton crop protection conference, pests and diseases. Survey, England: The British Crop Protection Council.

Chen, Z.X., and D.W. Dickson. 1998. Review of *Pasteuria penetrans*: Biology, ecology, and biological control potential. J. Nematol. 30:313-340.

Chen, Z.X., D.W. Dickson, R. Mc Sorley, D.J. Mitchell, and T.E. Hewlett. 1996. Suppression of *Meloidogyne arenaria* race 1 by soil application of endopsores of *Pasteuria penetrans*. J. Nematol. 28:159–168.

Chen, Z.X., D.W. Dickson, D.J. Mitchell, R. Mc Sorley, and T.E. Hewlett. 1997. Suppressive mechanisms of *Meloidogyne arenaria* race 1 by *Pasteuria penetrans*. J. Nematol. 29:1-8.

Chenault, K.D., M. Gallo, J.C. Seib, and V.A. James. 2007. A non-destructive seed sampling method for PCR-based analyses in marker assisted selection and transgene screening. Peanut Sci. 34:38-43.

Chen, X., F. Line, and H. Leung. 1998. Genome scanning for resistance-gene analogs in rice, barley, and wheat by high-resolution electrophoresis. Theor. Appl. Genet. 97:345-355.

Choi, K., M.D. Burow, G. Church, G. Burow, A.H. Patterson, C.E. Simpson, and J.L. Starr. 1999. Genetics and mechanism of resistance to *Meloidogyne arenaria* in peanut germplasm, J Nematol. 31:283-290.

Church, G. T., C. E. Simpson, M.D. Burow, A.H. Paterson, and J.L. Starr. 2000. Use of RFLP markers for identification of individuals homozygous for resistance to *Meloidogyne arenaria* in peanut. Nematology 2:575-580.

Church, G.M., and W. Gilbert. 1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA 88:1991-1995.

Chu, Y., C.C. Holbrook, P. Timper and P. Ozias-Akins. 2007. Development of a PCR-based molecular marker to select for nematode resistance in peanut. Crop Sci. 47:841-845.

Cliff, G., and H. Hirschmann. 1985. Evaluation of morphological variability in *Meloidogyne arenaria*. J. Nematol. 17:445-459.

Cobb, W.Y., and B.R. Johnson. 1973. Peanuts: Culture and uses, Stillwater Ok (ed). Am. Peanut Res. Educ. Soc. p. 42.

Concibidio, V.C., D.A. Lange, R.L. Denny, J.H. Orf, and N.D. Young. 1997. Soybean cyst nematode resistance genes in 'Peking', PI 90763, and PI 88788 using DNA markers, Crop Sci. 37:258-264.

Crouch, J.H. 2001. Molecular marker-assisted breeding: a perspective for small to medium-sized plant breeding companies. APSA Technical Report 30:1-14.

Davis, E.L., R.S. Hussey, T.J. Baum, J. Bakker, A. Schots, M.N. Rosso, and P. Abad. 2000. Nematode parasitism genes. Annu. Rev. Phytopathol. 38:365-96.

Davies, K.G., B.R. Kerry, and C.A. Flynn. 1988. Observations on the pathogenicity of *Pasteuria penetrans,* a parasite of root-knot nematodes. Annals Appl. Biol. 112:491-501.

Daykin, M.E., and R.S. Hussey. 1985. Staining and histopathological techniques in nematology. *In* K.R. Barker et al. (ed.). An advanced treatise on *Meloidogyne*. Vol. II: Methodology. North Carolina State Univ., Raleigh p. 39–48.

Day, I. S., V.S. Reddy, S. Ali, and A.S.N. Reddy. 2002. Analysis of EF-hand-containing proteins in *Arabidopsis*. Genome Biol. 3:1-24.

Dellaporta, S.J., J. Wood, and J.B. Hicks. 1983. A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. I.19-21.

Diachenko, L., F.C. Lau Y, A.P. Campbell, A. Chenchik, F. Mogadam, B. Huang, S. Lukyanov, K. Lukyanov, N. Gurskaya, E.D. Sverdlov, and P.D. Siebert. 1996. Suppression Subtractive Hybridization: A method for generating differentially regulated or tissue-specific cDNA probes and libraries. PNAS 93:6025-6030.

Diachenko, L., F.C. Lau Y, A.P. Campbell, A. Chenchik, F. Mogadam, B. Huang, S. Lukyanov, K. Lukyanov, N. Gurskaya, E.D. Sverdlov, and P.D. Siebert. 1996. Suppression Subtractive Hybridization: A method for generating differentially regulated or tissue-specific cDNA probes and libraries. PNAS 93:6025-6030.

Diatchenko, L., S. Lukyanov, F.C. Lau Y, and P.D. Siebert. 1999. Suppression subtractive hybridization: a versatile method for identifying differentially expressed genes. Methods Enzymol. 303:349-380.

Dickson, D. W. 1998. Peanut. *In* K.R. Barker, G.A. Peterson, and G.L. Windham (ed.). Plant and nematode interaction. Madison, WI: American Society of Agronomy, p. 523-566.

Dickson, D.W., and D. De Waele. 2005. Nematode parasites of peanut. Luc M., Sikora R. A., Bridge J. (ed.). Plant-parasitic nematodes in subtropical and tropical agriculture, 2nd ed. CAB International p. 393-436.

Dickson, D.W., and T.E. Hewlett. 1988. Efficacy of fumigant and nonfumigant nematicides for control of *Meloidogyne arenaria* on peanut. Annals Appl. Nemat. (J. Nematol. Vol. 20, Supplement) 2:95-101.

Dickson, D.W., and T.E Hewlett. 1989. Effects of bahiagrass and nematicides on *Meloidogyne arenaria* in peanut. J. Nematol. 21:671-676.

Dickson, D. W., D. J. Mitchell, T. E. Hewlett., M. Oostendorp, and M. E. Kannwischer-Mitchell. 1991. Nematode-suppressive soil from a peanut field. J. Nematol. 23:526.

Dickson, D.W., M. Oostendorp, R.M. Gibin-Davis and D.J. Mitchell. 1994. Control of plant parasitic nematodes by biological antagonists. In: Rosen, D., Bennett, F.D. and Capinera, J.L. (eds) Pest management in the subtropics. Biological Control –A Florida Perspective. Intercept, Andover, UK, pp.575-601.

Dixon, R.A., M.J. Harrison, and C.J. Lamb. 1994. Early events in the activation of plant defense responses. Annu. Rev. Phytopathol. 32:479-501.

Dixon, M.S.K., D.A. Jones, J.S. Keddie, C.M. Thomas, K. Harrison, and J.D. Jones. 1996. The tomato Cf-2 disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins. Cell 84:451–459.

Dixon, R.A., and C.J. Lamb. 1990. Molecular communication in interactions between plants and microbial pathogens. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41:339-367.

Donald, T., F. Pellerone, A.F. Adam-Blondon, A. Bouquet, M. Thomas, and I. Dry. 2002. Identification of resistance gene analogs linked to a powdery mildew resistance locus in grapevine. Theor. Appl. Genet. 104:610-618.

Dunn, R.A. 1988. Peanut nematode management. Nematology plant protection pointer 11, Institute of Food and Agricultural Sciences, University of Florida, Gainesville.

Dwivedi, S.L., S. Gurtu, S. Chandra, W. Yuejin, and S.N. Nigam. 2001. Assessment of genetic diversity among selected groundnut germplasm. 1: RAPD analysis. Plant Breed. 120:345-349.

Dwivedi, S.L., J.H. Crouch, S.N. Nigam, M.E. Ferguson, and A.H. Paterson. 2003. Molecular breeding of groundnut for enhanced productivity and food security in the semi-arid tropics: opportunities and challenges. Adv. Agron. 80:153-221.

Eisenback, J. D., and H. Hirschmann. 1980. Morphological comparison of *Meloidogyne* males by scanning electron microscopy. J. Nematol. 12:23-32.

Ellis, J., and D. Jones. 1998. Structure and function of proteins controlling strain-specific pathogen resistance in plants. Curr. Opin. Plant Biol. 1:288-293.

FAO. 2004. http://faostat.fao.org/faostat/collections?version=ext&hasbulk=0 &subset= agriculture.

Feinberg, A. P., and B. Vogeltein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132:6-13.

Feuillet, C., G. Schachermayr, and B. Keller. 1997. Molecular cloning of a new receptor-like kinase gene encoded at the Lr10 disease resistance locus of wheat. Plant J. 11:45-52.

Fry, S.C., R.C. Smith, K.F. Renwick, D.J. Martin, S.K. Hodge, and K.J. Matthews. 1992. Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. Biochem. J. 282:821-828.

Garcia, G.M., H.T. Stalker, C. Schroeder, and G. Kochert. 1996. Identification of RAPD, SCAR and RFLP markers tightly linked to nematode resistance genes introgressed from *Arachis cardenasii* into *Arachis hypogaea*. Genome 39:836-845.

Garcia, G.M., H.T. Stalker, and G. Kochert. 1995. Introgression analysis of an interspecific hybrid population in peanuts (*Arachis hypogaea* L.) using RFLP and RAPD markers. Genome 38:166-176.

Gorbet, D.W. 2000. Registration of 'SunOleic 97R' peanut. Crop Sci. 40:1190-1191.

Gorbet, D.W. 2007a. Registration of 'Hull' peanut. J. Plant Registrations 1:125-126.

Gorbet, D.W. 2007b. Registration of 'ANorden' peanut. J. Plant Registrations 1:123-124.

Gorbet, D.W., and D. A. Knauft. 1997. Registration of SunOleic® 95R peanut. Crop Sci. 37:1392.

Grundy, S.M. 1986. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol in man. New Engl. J. Med. 314:745-748.

Hagihara, T., H. Masaru, T. Yoji, and Y. Naoto. 2004. Cloning of soybean genes induced during hypersensitive cell death caused by syringolide elicitor. Planta 218:606-614.

Halward, T.M., H.T. Stalker, and G. Kochert. 1993. Development of an RFLP linkage map in diploid peanut species. Theor. Appl. Genet. 87:379-384.

Halward, T.M., H.T. Stalker, E. Larue, and G. Kochert. 1991. Genetic variation detectable with molecular markers among unadapted germplasm resources of cultivated peanut and related wild species. Genome 34:1013-1020.

Hammond-Kosack, K., and J. Jones. 1997. Plant disease resistance genes. Annu. Rev. Plant Physiol. Plant Molec. Biol. 48:575-607.

Hammond-Kosack, K.E., P. Silverman, l. Raskin, and J.D.G. Jones. 1996. Race-Specific elicitors of *Cladosporium fulvum* induce changes in cell morphology and the synthesis of ethylene and salicylic acid in tomato plants carrying the corresponding *Cf* disease resistance gene. Plant Physiol. 110:1381-1394.

He, G., and C.S. Prakash. 1997. Identification of polymorphic DNA markers in cultivated peanut (*Arachis hypogaea* L.). Euphytica 97:143–149.

Herselman, L., R. Thwaites, F.M. Kimmins, B. Courtois, P.J.A. Van der Merwe, and S.E. Seal. 2004. Identification and mapping of AFLP markers linked to peanut (*Arachis hypogaea* L.) resistance to the aphid vector of groundnut rosette disease. Theor. Appl. Genet. 109:1423-1433.

Holbrook, C.C., and J.P. Noe. 1992. Resistance to the peanut root-knot nematode (*Meloidogyne arenaria*) in *Arachis hypogaea*. Peanut Sci. 19:35-37.

Holbrook, C.C., J.P. Noe, D.W Gorbet, and M.G. Stephenson. 1995. Evaluation of peanut breeding lines with resistance to peanut root-knot nematode. Proceedings of American Peanut Research and Education Society, Charlotte, North Carolina, 11-14 July 1995, 27, p.24.

Hopkins, M.S., A.M. Casa, T. Wang, S.E. Michell, R.E. Dean, G.D. Kochert, and S. Kresovich. 1999. Discovery and characterization of polymorphic simple sequence repeats (SSRs) in peanut. Crop Sci. 39:1243-1247.

Horton, P., and K. Nakai. 1997. Better prediction of protein cellular localization sites with the k nearest neighbors classifier. Proc. Int. Conf. Intell. Syst. Mol. Biol. 5:147–152.

Hulbert, S.H., C.A. Webb, S.M. Smith, and Q. Sun. 2001. Resistance gene complexes: evolution and utilization. Annu. Rev. Phytopathol. 39:285-312.

Hussey, R.S., and K.R. Barker. 1973. A comparison of methods of collecting inocula for *Meloidogyne* species, including a new technique. Plant Dis. Rep. 57:1025-1028.

Isleib, G.T., H.E. Pattee, and F.G. Giesbrecht. 2004. Oil, sugar, and starch characteristics in peanut breeding lines selected for low and high oil content and their combining ability. Journal of Agric. Food Chem. 52:3165-3168.

Jacobs, J.M.E., H.J. Van Eck, K. Horsman, P.F.P. Arens, B. Verkerk-Bakker, E. Jacobsen, A. Pereira, and W.J. Stiekem. 1996. Mapping of resistance to the potato cyst nematode Globodera rostochiensis from the wild potato species *Solanum* vernei, Mol. Breed. 2:51–60.

Johal, G., and S. Briggs. 1992. Reductase activity encoded by the *HM1* disease resistance gene in maize. Science 258:985-987.

Johnson, A.W., N.A. Minton, T.B. Brenneman, G.W. Burton, A.K. Culbreath, G. J. Gascho, and S.H. Baker. 1999. Bahiagrass, Corn, Cotton Rotations, and Pesticides for Managing Nematodes, Diseases, and Insects on Peanut. J. Nematol. 31:191–200.

Jones, A.M., and J.L. Dangl. 1996. Logjam at the Styx: programmed cell death in plants. Trends Plant Sci. 1:114-119.

Jones, D.A., C.M. Thomas, K.E. Hammond-Kosack, P.J. Balint-Kurti, and J.D.G. Jones. 1994. Isolation of the tomato *Cf-9* gene for resistance to *Clasosporium fulvum* by transposon tagging. Science 266:789-793

Jung, C., D. Cai, and M. Kleine. 1998. Engineering nematode resistance in crop species. Trends Plant Sci. 3:266–271.

Kaloshian, I., D.A. Lawn, V.M. Williamson, B.B. Westerdahl, and G. Mayo. 1996. Resistance-breaking nematodes identified in California tomatoes. California Agriculture 50:18-19.

Kang, H.G., R.C. Foley, L. O. A. Sanchez, C. Lin, and K.B. Singh. 2003. Target genes for OBP3, a Dof transcription factor, include novel basic helix-loop-helix domain proteins inducible by salicylic acid. Plant J. 35:362–372.

Kobe, B., and J. Deisenhofer. 1994. The leucine-rich repeat: a versatile binding motif. Trends Biol. Sci. 19:415–421.

Kochert, G., T. Halward, W.D. Branch, and C.E. Simpson. 1991. RFLP variability in peanut (*Arachis hypogaea* L.) cultivars and wild species. Theor. Appl. Genet. 81:565-570.

Kokalis-Burelle, N., W.F. Mahaffee, R. Rodriguez-Kabana, J.W. Kloepper, and K.L. Bowen. 2002. Effects of switchgrass (*Panicum virgatum*) rotations with peanut (*Arachis hypogaea* L.) on nematode populations and soil microflora**.** J. Nematol. 34:98–105.

Kris-Etherton, P. M., T.A. Pearson, Y. Wan, R.L. Hargrove, K. Moriarty, V. Fishell, and T. Etherton. 1999. High-monunsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. Am. J. Clin. Nutr. 70:1009-1015.

Kyte, J., and R. Doolittle. 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 157:105-132.

Lagudah, E.S., O. Moullet, and R. Appels. 1997. Map based cloning of a gene sequence encoding a nucleotide binding domain and leucine rich region at the Cre3 nematode resistance locus of wheat. Genome 40:659–665.

Lamb, C. 1996. A ligand-receptor mechanism in plant-pathogen recognition. Science 274:2038-2039.

Logemann, J., J. Schell, and L. Willmitzer. 1987. Improved method for the isolation of RNA from plant tissues. Anal. Biochem. 163:16-20.

Lanham, P.G., S. Fennell, J.P. Moss, and W. Powell. 1992. Detection of polymorphic loci in *Arachis* germplasm using random amplified polymorphic DNAs. Genome 35:885-889.

Lawrence, G.J., E.J. Finnegan, M.A. Ayliffe, and J.G. Ellis. 1995. The *L6* gene for flax rust resistance is related to the *Arabidopsis* bacterial resistance gene *RPS2* and the tobacco viral resistance gene N. Plant Cell 7:1195-1206.

Marban-Mendoza, N., A. Jayaprakash, H.B. Jansson, R.A. Damon, and B.M. Zuckerman. 1987. Control of root-knot nematodes on tomato by lectins. J. Nematol 19:331–35.

Marcio, C. M., M.S. Hopkins, and S.E. Mitchell. 2004. Genetic diversity of peanut (*Arachis hypogaea* L.) and its wild relatives based on the analysis of hypervariable regions of the genome. BMC Plant Biol. 4:11.

Martin, G.B., S.H. Brommonschenkel, J. Chunwongse, A. Frary, and M.V. Ganal. 1993. Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432–1436.

Martin, G.B., J. Adam, Bogdanove, and G. Sessa. 2003. Understanding the functions of plant disease resistance proteins. Annu. Rev. Plant Biol. 54:23–61.

McSorley, R., D.W. Dickson, E.M. Candenado-Lay, T.E. Hewlett, and J.J. Frederick. 1992. Damage functions of *Meloidogyne arenaria* on peanut. J. Nematol. 24:193-198.

Melchinger, A.E. 1990. Use of molecular markers in breeding for oligogenic disease resistance. Plant Breed. 104:1-19.

Michelmore, R.W. 1996. Flood warning - resistance genes unleashed. Nature Genet. 14:376-378.

Milligan, S.B., J. Bodeau, J. Yaghoobi, I. Kaloshian, and P. Zabel. 1998. The root-knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. Plant Cell 10:1307-1319.

Mindrinos, M., F. Katagiri, G.L. Yu, and F.M. Ausubel. 1994. The *A. thaliana* disease resistance gene *RPS2* encodes a protein containing a nucleotide-binding site and leucine-rich repeats. Cell 78:1089-99.

Minton, N.A., and R.M. Sayre. 1989. Suppressive influence of *Pasteuria penetrans* in Georgia soils on reproduction of *Meloidogyne arenaria*. J. Nematol. 21:574-575.

Moore, K. M., and D.A. Knauft. 1989. The inheritance of high oleic acid in peanut. J. Hered. 80:252-253.

Nachin, L., U. Nannmark, and T. Nystrom. 2005. Differential roles of the universal stress proteins of *Escherichia coli* in oxidative stress resistance, adhesion, and motility.J. Bacteriol. 187:6265-6272.

Nelson, S.C., C.E. Simpson, and J.L. Starr. 1989. Resistance to *Meloidogyne arenaria* in *Arachis* species germplasm. Supplement to the J. Nematol. 21:654-660.

Nelson, S.C., J.L. Starr, and C.E. Simpson. 1990. Expression of resistance to *Meloidogyne arenaria* in *Arachis batizocoi* and *A. cardenasii*. J.Nematol. 22:423-425.

Niewöhner, J., F. Salamini, and C. Gebhardt. 1995. Development of PCR assays diagnostic for RFLP marker alleles closely linked to alleles *Gro1* and *H1*, conferring resistance to the root cyst nematode *Globodera rostochiensis* in potato. Mol. Breed. 1:65-78.

Nishitani, K., and R. Tominaga. 1991. In vitro molecular weight increase in xyloglucans by an apoplastic enzyme preparation from epicotyls of *Vigna angularis*. Physiol. Plant. 82:490-497.

Noe, J.P., C.C. Holbrook, and N.A. Minton. 1992. Field evaluation of susceptibility to *Meloidogyne arenaria* in *Arachis hypogaea* plant introductions. Supplement to the J. Nematol. 24:712-716.

Norden, A. J., D.W. Gorbet, D.A. Knauft, and C.T. Young. 1987. Variability in oil quality among peanut genotypes in the Florida breeding program. Peanut Sci. 14:7-11.

Norden, A.J., V.G. Perry, F. G. Martin, and J. NeSmith. 1977. Effect of age of bahiagrass sod on succeeding peanut crops. Peanut Sci. 4:71-74.

Nystrom, T., and F.C. Neidhardt. 1994. Expression and role of the universal stress protein, UspA, of *Escherichia coli* during growth arrest. Mol. Microbiol. 11:537-544.

O'Byrne, D.J., D.A. Knauft, and R.B. Shireman. 1997. Low fat–monounsaturated rich diets containing high-oleic peanuts improve serum lipoprotein profiles. Lipids 32:687-695.

O'Keefe, S. F., V.A. Wiley, and D.A. Knauft. 1993. Comparison of oxidation stability of high- and normal-oleic peanut oils. J. Am. Soc. Oil Chem.70:489-492.

Oostendorp, M., D.W. Dickson, and D.J. Mitchell. 1991. Population development of *Pasteuria penetrans* on *Meloidogyne arenaria.* J. Nematol. 23:58-64.

Ori, N., Y. Eshed, I. Paran, G. Presting, D. Aviv, S. Tanksley, D. Zamir, and R. Fluhr. 1997. The I2C family from the wilt disease resistance locus *I2* belongs to the nucleotide binding, leucine-rich repeat superfamily of plant resistance genes. Plant Cell 9:521-532.

Oshimura, S., U. Yamanouchi, Y. Katayose, S. Toki, Z.X. Wang, I. Kono, N. Kurata, M. Yano, N. Iwata, and T. Sasaki. 1998. Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. Proc. Natl. Acad. Sci. USA 95: 1663-1668.

Paik-Ro, O.G., R.L. Smith, and D.A. Knauft. 1992. Restriction length polymorphism evaluation of six peanut species within the *Arachis* section. Theor. Appl. Genet. 84:201-208.

Paterson, A.H., H.T. Stalker, M. Gallo, M.D. Burow, S.L. Dwivedi, J.H. Crouch, and E.S. Mace. 2004. Genomics and genetic enhancement of peanut. In *Legume Crop Genomics*, edited by R.F. Wilson, H.T. Stalker, E.C. Brummer, AOCS press, Champaign, Illinois, 97-109.

Qui, J., J. Hallmann, N. Kokalis-Burelle, D.B. Waever, R. Rodríguez-Kábana and S. Tuzun. 1997. Activity and differential induction of chitinase isozymes in soybean cultivars resistant or susceptible to root-knot nematodes. J. Nematol. 29:523-530.

Rebrikov, D.V., O.V. Britanova, N.G. Gurskaya, K.A. Lukyanov, V.S. Tarabykin, and S.A. Lukyanov. 2000. Mirror orientation selection (MOS): a method for eliminating false positive clones from libraries generated by suppression subtractive hybridization. Nucleic Acids Res. 28:E90.

Rodriguez-Kabana, R. and H. Ivey. 1986. Crop rotation systems for the management of *Meloidogyne arenaria* in peanut. Nematropica 16: 53-63.

Rodriguez-Kabana, R., C.F. Weaver, D.G. Robertson, and E.L. Snoddy. 1986. Population dynamics of *Meloidogyne arenaria* juveniles in a field with Florunner peanuts. Nematropica. 16:185-196.

Sabine, L., O. Doring, S. Heuer, H. Luthen, and M. Bottger. 1997. Oxidoreductases in plant plasma membranes. Biochimica et Biophysica Acta (BBA) - Reviews on Biomembranes 131:81-102.

Sambrook, J., and D.W. Russell. 2001. Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Sano, T., and T. Nagata. 2002. The possible involvement of phosphate induced transcription factor encoded by *Phi-2* gene from tobacco in ABA signaling pathways. Plant Cell Physiol. 43:12-20.

Sasser, J.N. 1954. Identification and host-parasite relationships of certain root-knot nematodes (*Meloidogyne* spp.). Univ. Maryland Agric. Exp. Stn. Bull A-77:1-13.

Sasser, J. N. 1982. Relative importance and frequency of occurrence of various species, pathogenic variation and host races. *In* Proceedings of the IMP research and planning conference on root-knot nematodes, *Meloidogyne* spp. (Region III).Brazil. Raleigh: North Carolina State University Graphics. p. 162-163.

Sasser, J.N. 1990. Plant-parasitic Nematodes: The Farmer's Hidden Enemy. North Carolina State University Press, Raleigh, NC. p. 47–48.

Sasser, J.N., and C.C. Carter. 1982. Root-knot nematodes (*Meloidogyne* spp.): Identification, morphological and physiological variation, host range, ecology, and control. *In* R. D. Riggs (ed.). Nematology in the southern region of the United States. Arkansas Agric. Exp. Sta., South. Coop. Series Bull. 276. p. 21-32.

Sasser, J.N., and D.W. Freckman. 1987. A world perspective on nematology: the role of society. In: Veech, J.A. and Dickson, D.W. (eds), *Vistas on Nematology*. A commemoration of the 25th Anniversary of the Society of Nematologists. Society of Nematologists Inc, Hyattsville, MD, USA, pp 7-14.

SAS Institute. 1985. SAS user's guide. SAS Institute, Cary, NC.

Sayre, R.M., and M.P. Starr. 1985. *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n, sp. n., a mycelial and endospore-forming bacterium parasitic in plant parasitic nematodes. Proc. Helminthological Society of Washington. 52:149-165.

Seah, S., H. Bariana, J. Jahier, Sivasithamparam, and E.S. Lagudah. 2001. Cloning and characterization a family of disease resistance gene analaogs from wheat and barley. Theor. Appl. Genet. 97:937-945.

Sharma, K.K., M. Lavanya, and V. Anjaiaha. 2000. A method for isolation and purification of peanut genomic DNA suitable for analytical applications. Plant Mol. Biol. Rep. 18:393a-393h.

Sharp, R.E., Y. Wu, G.S. Voetberg, I.N. Saab, and M.E. LeNoble. 1994. Confirmation that abscisic acid accumulation is required for maize primary root elongation at low water potentials. J. Exp. Bot. 45:1743-1751.

Simpson, C.E. 1991. Pathways for introgression of pest resistance into *Arachis hypogaea* L. Peanut Sci. 18:22-26.

Simpson, C.E., S.C. Nelson, J.L. Starr, K.E. Woodard, and O.D. Smith. 1993. Registration of TxAG-6 and TxAG-7 peanut germplasm lines. Crop Sci. 33:1418.

Simpson, C.E., and S.C. Starr. 2001. Registration of 'COAN' peanut. Crop Sci. 4:918.

Simpson, C.E., S.C. Starr, G.T. Church, M.D. Burow, and A.H. Paterson. 2003. Registration of 'NemaTAM' peanut. Crop Sci. 43:1561.

Smith, R.C., and S.C. Fry. 1989. Extracellular transglycosylation involving xyloglucan oligosaccharides in vivo. In SC Fry, CT Brett, JSG Reid, eds, Fifth Cell Wall Meeting Book of Abstracts and Programme. Scottish Cell Wall Group, Edinburgh, p 139.

Song, W.Y., G.L. Wang , L.L. Chen, H.S. Kim, L.Y. Pi, T.E. Holsten, J. Gardner, B. Wang, W.X. Zhai, L.H. Zhu, C. Fauquet, and P.C. Ronald. 1995. A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science 270:1804-1806

Soriano, J.M., S. Vilanova, C. Romero G. Lla´ cer, and M.L. Badenes. 2005. Characterization and mapping of NBS-LRR resistance gene analogs in apricot (*Prunus armeniaca* L.) Theor. Appl. Genet.110:980-989.

Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-517.

St. Angelo, A.J., and R.L. Ory. 1973. Investigations of causes and prevention of fatty acid peroxidation in peanut butter. J. Am. Peanut Res Educ. Assoc. 5:128-133.

Starr, J.L., and C.E. Simpson. 1991. Segregation for resistance to *Meloidogyne arenaria* in progeny of interspecific hybrids. Proc. Am. Peanut Res. & Ed. Soc. 23:23.

Stalker, H.T., and J.P. Moss. 1987. Speciation, cytogenetics, and utilization of Arachis species. Adv. Agron. 41:1–40.

Stalker, H.T., T.D. Phillips, J.P. Murphy, and T.M. Jones. 1994. Variation of isozyme patterns among Arachis species. Theor. Appl.Genet. 87:746-755.

Starr, J.L., E.R. Morgan, and C.E. Simpson. 2002. Management of peanut root-knot nematode, *Meloidogyne arenaria*, with host resistance. Online. Plant health progress dol:10.1094/php-2002-1221-01-HM.

Starr, J.L., C.E. Simpson, and T.A. Lee. 1995. Resistance to *Meloidogyne arenaria* in advanced generation breeding lines of peanut. Peanut Sci. 22:59-61.

Sturgeon, R.V. 1986. Peanut disease loss estimates for major peanut producing states in the United Sates in 1984 and 1985. Proc. Am. Peanut Res. Educ. Soc. 18:24-26.

Subramanian, V., S. Gurtu, R.C.N. Rao, and S.N. Nigam. 2000. Identification of DNA polymorphism in cultivated peanut using random amplified polymorphic DNA (RAPD) assay. Genome 43:656-660.

Takken, F.L.W., and M.A.H.J. Joosten. 2000. Plant resistance genes: their structure, function and evolution. Eur. J. Plant Pathol. 106:699-713.

Tameling, W.I.L., S.D. Elzinga, P.S. Darmin, J.H. Vossen, F.L.W. Takken, M.A. Haring, and B.J.C. Cornelissen. 2002. The tomato *R* gene products I-2 and Mi-1 are functional ATP binding proteins with ATPase activity. Plant Cell 14:2929-2939.

Tanksley, S.D., N.D. Young, A.H. Paterson, and M.W. Bonierbal. 1989. RFLP mapping in plant breeding: new tools for an old science. Bio/Technol. 7:257-264.

Taylor, A.L. 2003. Nematocides and nematicides - a history. Nematropica 33:225-232.

Thurau, T., S. Kifle, C. Jung, and D. Cai. 2003. The promoter of the nematode resistance gene *Hs1pro-1* activates a nematode-responsive and feeding site-specific gene expression in sugar beet (*Beta vulgaris* L.) and *Arabidopsis thaliana*. Plant Mol. Biol. 52:643-660.

Traut, T.W. 1994. The functions and consensus motifs of nine types of peptide segments that form different types of nucleotide binding-sites. Eur. J. Biochem. 222:9-19

Van den Ackerveken, G.F.J.M., J.A.L. Van Kan, and P.J.G.M. de Wit. 1992. Molecular analysis of the avirulence gene *avr9* of the fungal tomato pathogen *Cladosporium fulvum* fully supports the gene-for-gene hypothesis. Plant J. 2:359–366.

Van der Vossen, E.A., J.N. Van der Voort, K. Kanyuka, A. Bendahmane, and H. Sandbrink. 2000. Homologues of a single resistance-gene cluster in potato confer resistance to distinct pathogens: A virus and a nematode. Plant J. 23:567–576.

Weibelzahl-Fulton, E. 1998. Suppression of *Meloidogyne* spp. by *Pasteuria penetrans*. Ph.D. dissertation, University of Florida, Gainesville.

Whitham, S., S.P. Dinesh-Kumar, D. Choi, R. Hehl, C. Corr, and B. Baker. 1994. The product of the tobacco mosaic virus resistance gene *N*: Similarity to Toll and the Interleukin-1 receptor. Cell 78:1101-1115.

Williamson, Y.V.M. 1998. Root-knot nematode resistance genes in tomato and their potential for future use. Annu. Rev. Phytopathol. 36:277–293

Wu, Y., W.G. Spollen, R.E. Sharp, P.R. Hetherington, and S.C. Fry. 1994. Root growth maintenance at low water potentials. Plant Physiol.106:607-615.

Young, N.D. 2000. The genetic architecture of resistance. Curr. Opin. Plant Biol. 3:285-290.

Yuksel, B., J.C. Estill, S.R. Schulze, and A.H. Paterson. 2005. Organization and evolution of resistance gene analogs in peanut. Mol. Genet. Genom. 274:248-263.

Zhu, Y.Y., E.M. Machleder, A. Chenchik, R. Li, and P.D. Siebert. 2001. Reverse transcriptase template switching: a SMART approach for full-length cDNA library construction. Biotechniques 30:892-897.

Zijlstra, C., D. Donkers-Venne, and M. Fargette. 2000. Identification of *Meloidogyne incognita, M. javanica* and *M. arenaria* using sequence characterized amplified region (SCAR) based PCR assays. J. Nematol. 2:847-853.

Zinoveva, S.V., E.A. Perekhod, A.V. Ilina, Z. Udalova, N.G. Gerasimova , N.I. Vasyukova, O.L. Ozeretskovskaya, and M.D. Sonin. 2001. PR proteins in plants infected with the root-knot nematode *Meloidogyne incognita*. Dokl. Biol. Sci. 379:393-3955.

Zinoveva, S.V., N. I. Vasyukova, and O.L. Ozeretskovskaya. 2004. Biochemical aspects of plant interactions with phytoparasitic nematodes: A review. Appl. Biochem. Microbiol. 40:133–142.

BIOGRAPHICAL SKETCH

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