

Development of a PCR-Based Molecular Marker to Select for Nematode Resistance in Peanut

Y. Chu, C. C. Holbrook, P. Timper, and P. Ozias-Akins*

ABSTRACT

The peanut root-knot nematode [*Meloidogyne arenaria* (Neal) Chitwood race 1] is a significant pathogen on peanut (*Arachis hypogaea* L.). Nematode resistant cultivars would reduce yield losses while reducing the use of nematicides in fields where these nematodes occur. Through years of breeding effort, nematode resistance gene(s) have been introgressed into peanut cultivars from their wild relatives, *Arachis* spp. Molecular markers RKN440 and Z3/265, linked to the resistance gene, previously were identified by random amplified polymorphic DNA (RAPD) analysis. Unfortunately, when these markers were applied to our breeding programs, neither could give a reproducible level of correlation with the phenotype data. In this study, we modified the marker RKN440 based on more complete sequencing data and established a new nematode resistance dominant marker 197/909. This marker is reproducible and shows a high correlation with the phenotype data. It amplifies fragments from both susceptible and resistant samples, but of different molecular weights, avoiding false negative judgment caused by failed reactions with dominant markers. When we applied this marker using a cost-effective, high-throughput DNA extraction method, it remained a robust assay. Plant breeders will be able to use this new marker to hasten efforts to combine nematode resistance with other important characteristics in peanuts.

Y. Chu and P. Ozias-Akins, Dep. of Horticulture, Univ. of Georgia Tifton Campus, Tifton, GA 31793; C.C. Holbrook and P. Timper, USDA ARS, P.O. Box 748, Tifton, GA 31793. Funding was provided by the Georgia Seed Development Commission and the University of Georgia Research Foundation Cultivar Development Program. Received 17 July 2006.
*Corresponding author (pozias@uga.edu).

Abbreviations: RAPD, random amplified polymorphic DNA; SCAR, sequence characterized amplified region.

IN THE SOUTHERN USA, root-knot nematodes cause significant reductions in peanut yield. It has been estimated that in heavily infested fields, peanut production is less than half that of the non-infested fields (Minton and Baujard, 1990). In Georgia, this disease causes \$16.4 million loss each year. Crop rotation and nematicides are commonly used to reduce losses from this nematode. Rotating nonhost crops with peanut can reduce populations of *M. arenaria* and crop damage (Johnson et al., 1999; Rodriguez-Kabana et al., 1995); however, because of the wide host range of this nematode, few marketable nonhost crops are available in the United States. The application of nematicides has been limited due to governmental regulations and environmental concerns. The use of nematode resistant peanut cultivars would reduce yield losses and use of nematicides in fields where these nematodes occur.

Introgression of root-knot nematode resistance genes into peanut cultivars could be a valuable approach to control this disease (Simpson, 2001). Most peanut cultivars are susceptible to nematodes and only moderate levels of resistance to nematodes have been observed in *A. hypogaea* germplasm collections (Holbrook et

Published in *Crop Sci* 47:841–847 (2007).

doi: 10.2135/cropsci2006.07.0474

© Crop Science Society of America

677 S. Segoe Rd., Madison, WI 53711 USA

All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Permission for printing and for reprinting the material contained herein has been obtained by the publisher.

al., 2000, 1998). However, high levels of resistance have been discovered among the related *Arachis* spp. (Baltensperger et al., 1986; Holbrook and Noe, 1990; Nelson et al., 1989, 1990). In the past decade, introgression of nematode resistance genes from *Arachis* spp. resulted in the release of nematode resistant cultivars and germplasms such as COAN (Simpson and Starr, 2001), GP-NC WS 5 and GP-NC WS 6 (Stalker et al., 2002a), NemaTAM (Simpson et al., 2003), and NR0812 and NR0817 (Anderson et al., 2006). These peanut germplasms have been further used in breeding programs to achieve multiple disease control, as well as yield and quality improvement (Holbrook et al., 2003; Starr et al., 1995). To assist the breeding process, two RAPD markers tightly linked to nematode resistance gene(s), Z3/265 (Garcia et al., 1996) and RKN440 (Burow et al., 1996), were converted into sequence characterized amplified region (SCAR) markers. Marker Z3/265 was developed from an F₂ population of GA6 (*A. hypogaea* [PI 261942] × *A. cardenasii*) backcrossed with PI261942. This 265-bp fragment derived from *A. cardenasii* was linked at 10 ± 2.5 cM and 14 ± 2.9 cM from the putative nematode resistance genes *Mag* and *Mae* respectively (Garcia et al., 1996). Our application of Z3/265 repeatedly resulted in a high rate of false positive results (data not shown). It is difficult to modify this marker due to the small size of the amplified fragment. Therefore, we focused our research effort on improving marker RKN440. Marker RKN440 was discovered in a diploid interspecific program involving crosses among *A. cardenasii*, *A. diogeni* Hoehne, and *A. batizocoi* Krapov. and W.C. Gregory with *A. hypogaea* cv. Florunner which generated the nematode resistant line TXAG-7. Marker RKN440 was identified in the backcross population with a 5.8 ± 2.1% recombination rate with the resistance gene (Burow et al., 1996). Partial sequences of the RAPD fragment were deposited in GenBank (U65587, U65588) and allowed primers for a SCAR marker to be designed (data not shown). Contrary to SCAR Z3/265, SCAR RKN440 showed a high rate of false negative identification in our breeding programs. The objective of this research was to develop a new molecular marker based on RKN440 which reproducibly correlated with phenotypic data. We then tested the feasibility of using this new marker in a high throughput screening mode. Application of the new marker with the high throughput DNA extraction method could dramatically reduce the time and cost of a nematode resistance breeding program.

MATERIALS AND METHODS

Plant Materials

Two tests were performed to assay for nematode resistance and marker genotype. The first was conducted with a group of advanced breeding lines derived from crossing nematode resistant parents with various susceptible parents while the second was conducted with F_{2,3} families from a single cross.

Advanced nematode resistant lines tested in this study were F₇ breeding lines from crosses of resistant parent COAN and various susceptible parents C99R, Virugard, FLA MDR 98, and Georgia Green. In addition, two more advanced lines were from the F₇ generation of GP-NC WS6 × C99R and GP-NC WS5 × C99R. Initial selection for nematode resistance was made in the F₃ generation. Additional individual plant selections were made in the F₅ generation. All advanced breeding lines were planted in the greenhouse in a randomized complete block design with four replications per line to evaluate nematode resistance and the presence of molecular markers.

The segregating population consisted of F₃ plants from 84 F₂ families (NemaTAM × GP-NC WS14). The susceptible parent GP-NC WS14 has been shown to be resistant to leaf spot disease [early leaf spot disease caused by *Cercospora arachidicola* Hori and late leaf spot disease caused by *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton] (Stalker et al., 2002b). Five seeds from each F₂ family were planted in the greenhouse. This was the first round of nematode resistance screening since the F₂ plants were harvested without selection. Therefore, in this study, we pooled the F₃ tissues for DNA extraction to reconstruct the F₂ genotypes. Selected F₃ individuals were also tested by molecular markers.

Nematode Resistance Test

The nematode resistance tests for the advanced breeding lines and the segregating population were essentially the same except for slight differences in the rating methods. In both tests, pots containing a single peanut plant were inoculated with 8000 eggs of *M. arenaria* 2 wk after planting. The pots were completely randomized in the greenhouse where soil temperatures varied between 20 and 35°C. Nematode eggs used for inoculum were extracted from tomato (*Solanum lycopersicum* L.) roots with 0.5% NaOCl (Hussey and Barker, 1973). For the advanced lines, all four replicates were uprooted and rinsed 56 d after inoculation. The roots were stained with 0.05% phloxin B solution for 3 to 5 min. Each plant-root system was rated according to the number of egg masses and galls with the following scale: 0 = no galls or no egg masses, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100 and 5 = more than 100 galls or egg masses per root system. If the egg mass count was ≤1 and the gall count was ≤2, the plant was classified as phenotypically resistant, otherwise, it was regarded as susceptible to nematode reproduction (Holbrook et al., 2003).

For the segregating population, the five replicates were uprooted 61 d after inoculation. Nematode eggs were extracted from roots by cutting the entire root system into approximately 5-cm pieces, placing the pieces in a 1-L flask, and agitating for 4 min in a 1% NaOCl solution (Hussey and Barker, 1973). Eggs were collected and rinsed with tap water on nested 150- and 25-μm-pore sieves, and counted using a dissecting microscope. Individual plants were considered resistant if nematode reproduction (eggs per pot) was less than 10% of the average reproduction on the 10 most susceptible progeny (Hussey and Janssen, 2002). Based on these criteria, the susceptible parent could not be used as a standard susceptible control because it showed moderate resistance to *M. arenaria* reproduction. Nevertheless, the moderate resistance in GP-NC WS14 did not obscure the analysis of resistance among population extremes due to the major-effect locus in NemaTAM. For the entire

segregating population, correlation analysis was performed between $F_{2,3}$ average egg counts and their genetic marker scores (present = 1, absent = 0). Correlation analysis was also performed on the F_3 individual egg counts and their respective genetic marker scores. All statistical analyses were performed with SAS 9.1 (SAS Institute Inc., Cary, NC).

DNA Extraction and PCR Amplification

Partially unfolded, healthy leaflets were collected from each plant and ground in liquid N_2 . For the segregating population, leaflets (~250 mg) from five progenies within the same $F_{2,3}$ family were pooled to reconstruct the genotype of the F_2 parents. Pulverized tissues were extracted by the DNeasy 96 plant kit following the manufacturer's instructions (QIAGEN, Valencia, CA). A high-throughput DNA extraction method (Xin et al., 2003) also was applied to individuals from 16 families of the segregating population. Briefly, a 6-mm leaf disk was punched from each sample plant and placed in a 96-well Uniplate (Whatman, Clifton, NJ), and 50 μ L of buffer A (100 mM NaOH, 2% Tween 20) was added to each well. The plate was incubated at 95°C for 10 min then 50 μ L of buffer B (100 mM Tris-HCl, 2 mM EDTA) was added to each well. After thorough mixing, 2 μ L of the extract was used for a 25- μ L volume PCR amplification. Each PCR amplification was performed with 0.5 U of Hotmaster *Taq* DNA polymerase (Eppendorf, Hamburg, Germany) using the buffer supplied by the manufacturer containing 25 mM Mg^{2+} (final reaction concentration, 2.5 mM). With the high-throughput DNA extracts, PVP-40 (Sigma, St. Louis, MO) and bovine serum albumin (New England Biolabs, Hertfordshire, UK) were added to the reaction mix at final concentrations of 4% and 20 μ g μ L⁻¹, respectively. Amplification conditions for all sets of primers were similar: initial denaturation at 94°C for 5 min; 40 cycles of 94°C for 30 s, annealing temperature for 30 s, and 72°C for 30 s; final extension at 72°C for 7 min. The annealing temperature of all primer combinations was 60°C except for 198/911 primer pair whose annealing temperature was 36°C. All PCR amplifications were performed with the GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA).

Cloning and Sequencing PCR Amplicons

PCR products were separated on 0.8% agarose gels (Invitrogen, Carlsbad, CA). Bands of desired molecular weight were excised and purified using a QIAGEN gel purification kit. DNA concentration was determined by fluorometry. Gel-purified fragments were ligated with the pGEM-Easy TA cloning vector (Promega, Madison, WI). Ligated plasmids were transformed into *E. coli* DH5 α competent cells (Invitrogen) via the heat-shock method. Sequencing of inserts was performed with a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA). Vector NTI software program (Invitrogen) was used for primer design and sequence analysis.

RESULTS AND DISCUSSION

Application of marker RKN440 for screening nematode resistant progenies generated a high frequency of false negative classifications. Screening results on the advanced

nematode resistant lines showed that only 44 of 95 (46.3%) nematode resistant plants were amplifiable with RKN440 primers. The low rate of amplification within the resistant group disqualified RKN440 as an effective selective marker for nematode resistance. Meanwhile, RKN440 amplified only 1 out of 25 (4.0%) susceptible plants. It has been reported that RKN440 has 5.8% recombination with nematode resistance (Burow et al., 1996); therefore, a low frequency of resistant lines lacking the marker or susceptible lines containing the marker would be expected.

The sequence of RKN440 previously reported (Burow et al., 1996), consisted of only two ~200-bp-long fragments starting at the RAPD primer sites (GenBank IDs U65587, U65588). The RAPD marker was reported to be ~600 bp in size and our amplicon size, which was designed to span the entire RAPD fragment, was around 650 bp; therefore, more than 1/3 of the internal portion of the DNA sequence was unavailable. To design a more robust SCAR marker, one of the original sense or antisense primers was retained while searching for a compatible primer on the complementary DNA strand. Unfortunately, no new primer could be generated from the available sequence due to stretches of AT-rich regions (U65587: AT = 59.8%; U65588: AT = 83.0%). We therefore sequenced the RKN440 amplicon generated with COAN as the template (GenBank ID QD198152). Alignment of this sequence with the two published fragments, U65587 and U65588, showed 100% identity. Four primers were designed using full-length sequence of the RKN440 amplicon (Table 1). Two of them (no. 909 and 910) paired with SCAR RKN440 sense primer (no. 197) and the other two (no. 911 and 912) paired with SCAR RKN440 antisense primer (no. 198). All four new combinations could amplify both COAN and Georgia Green. Although primers 198/912 produced a very faint band with COAN which was reproducible, no size difference was observed between amplicons from COAN and Georgia Green (data not shown). Primer set 198/911 also did not produce any amplicon size difference between COAN and Georgia Green. The amplified products from 197/909 and 197/910 appeared to have small size differences after electrophoresis on a 0.8% agarose gel. Upon further separation on a 5% polyacrylamide gel, only 197/909 primer set demonstrated a true size difference (Fig. 1). The amplification reactions from COAN and Georgia Green also were mixed and heated to 95°C for 5 min followed by chilling to 4°C, then run on the same gel. The mixture showed that the lower band almost disappeared, whereas the higher bands were enhanced. These data suggest that the approximately 300-bp PCR products from both COAN and Georgia Green can form heteroduplexes. The smaller band amplified from Georgia Green with 197/909 primers was sequenced and aligned with the complete RKN440 sequence amplified from COAN. The 197/909 amplicon from Georgia Green

Table 1. Primer sequences. Combination of no. 197/198 produces the original SCAR RKN440.

Primer no.	Sequence	GenBank ID
197	5'-ctgtcgaacctggaagaagatcc-3'	U65587
198	5'-ctgtcgaaccggttaagtcgggtg-3'	U65588
909	5'-cacacttgatggtagaagtatgctt-3'	QD198152
910	5'-gtgactttgaaataaaataaattgaatataagtg-3'	QD198152
911	5'-cgaacaacgcta-3'	QD198152
912	5'-gaactaggtcagggttcgaacg-3'	QD198152

showed a 29-bp deletion starting from nucleotide position 60, a 3-bp deletion starting from nucleotide position 212, and a 1-bp insertion at nucleotide position 125. The 31-bp difference in size causes the band shift between the amplicons from Georgia Green and COAN. Besides the deletions, there are 11 single nucleotide polymorphisms between these two sequences.

Since it has been suggested that *A. hypogaea* was domesticated from diploids *A. duranensis* Krapov. and W.C. Gregory (A genome donor) and *A. ipaensis* Krapov. and W.C. Gregory (B genome donor) (Jung et al., 2003; Ramos et al., 2006; Seijo et al., 2004), we further checked the origin of this “susceptible” marker band. Using genomic DNA extracted from *A. duranensis* and *A. ipaensis*, only *A. duranensis* gave a band at the same size as that of Georgia Green, whereas *A. ipaensis* was not amplifiable with this primer set (data not shown). These data suggest that the fragment amplifiable with 197/909 in Georgia Green likely originated from the A subgenome rather than the B subgenome, and may be allelic to the “resistance” marker that is presumed to have originated from the A-genome species, *A. cardenasii* Krapov. and W.C. Gregory. The A-genome-derived susceptible marker is not detectable in heterozygotes that also contain the resistance marker probably because of preferential amplification of the resistance marker. We did not attempt to optimize reaction conditions that might permit both amplicons to amplify _

Population Screening with 197/909 Primers

Since polyacrylamide gel separation involves handling toxic gel mix and the number of samples that can be loaded on each gel is very limited, it is not a practical system for large-scale screening in a breeding program. Agarose gel is a safe separation system; it also can accommodate 250 samples on each gel. When the segregating popula-

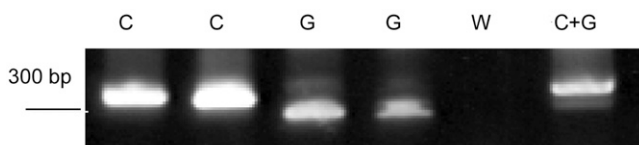


Figure 1. A 5% polyacrylamide gel separation of amplicons from COAN and Georgia Green with Primers 197/909. C, COAN; G, Georgia Green; W, water negative control; C+G, mixed and denatured products from COAN and Georgia Green.

tion was screened with 197/909, all 84 $F_{2,3}$ families were amplifiable, and the amplicons from susceptible and resistant plants could be accurately scored by size difference on 2% agarose gels (Fig. 2). Sixteen $F_{2,3}$ families (19%) had the marker which predicts susceptibility. Two resistant $F_{2,3}$ and three susceptible $F_{2,3}$ families, according to marker genotype, were selected to confirm the scoring by screening individual F_3 plants within a family (Fig. 3). In the resistant families, 3 out of 10 F_3 plants had the susceptible marker indicating that their F_2 parents were heterozygous. In the susceptible families, all the F_3 individuals showed the marker expected from a susceptible genotype. Therefore, the screening data for the reconstructed $F_{2,3}$ population can be confirmed with F_3 individual data.

The inoculation phenotype data based on nematode egg production were compared with the marker predictions using average egg numbers for $F_{2,3}$ families and their respective marker data. Average egg numbers ranged from 0 to 121 920 across $F_{2,3}$ families. Correlation analysis was conducted between the genetic maker scores and average egg number of the $F_{2,3}$ families. The result showed a significant negative correlation ($r = -0.64$, $P < 0.0001$) which means that the genotype score was inversely correlated with the average egg number. Since this analysis took into account both heterozygous and homozygous $F_{2,3}$ families, the correlation probably would have been stronger if heterozygous families were not included in the analysis. Families with an average egg number <7985 (10% of the egg count from the top 10 uniformly susceptible families) were considered to be highly resistant. The resistant parent NemaTAM averaged 600 eggs per plant whereas the susceptible parent GP-NC WS14 averaged 18 120 eggs. Based on the average egg production, 1 out of 33 (3%) highly resistant $F_{2,3}$ families did not amplify with the resistance marker. Out of 14 $F_{2,3}$ families that were uniformly susceptible, two (14.3%) amplified the resistance marker. Therefore, among the highly resistant and uniformly susceptible classes, only 6.4% (3/47) would have had erroneously predicted phenotypes based on marker genotype.

We also screened the F_3 individuals from eight resistant and eight susceptible $F_{2,3}$ families using a high-throughput DNA extraction method. Data from samples processed with the high-throughput extraction method were 100% correlated with the scores from DNeasy-extracted samples. Phenotype data for these individual plants exhibited an 82.8% match with genotype data. Of the resistant phenotypes, 6.3% of the plants would have been discarded based on their susceptible marker score. Of the susceptible phenotypes, 10.9% would have been selected as resistant based on their marker genotype. Correlation analysis of the marker genotype and the individual egg count demonstrated similar significant correlation with $r = -0.65$ ($P < 0.0001$).

In the advanced breeding lines, a subset of samples were screened with 197/909 primers. This set of prim-

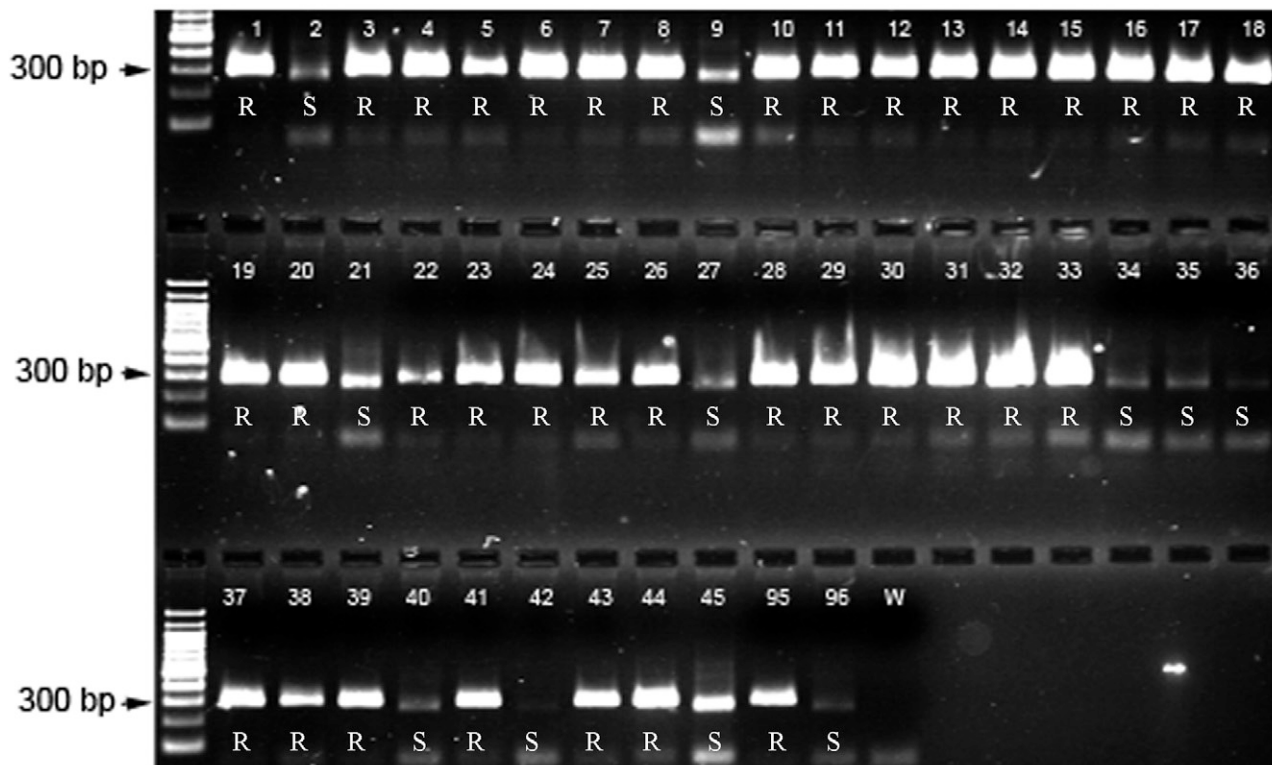


Figure 2. Screening of the segregating population for marker RKN440 (primer 197/909). No. 1 to 45 in $F_{2.3}$ families are loaded on 2% agarose gel. No. 95, NemaTAM parent; no. 96, GP-NC WS14 parent; W, water negative control; R, resistant; S, susceptible.

ers amplified the resistance marker in 87% of the resistant progenies (egg mass index ≤ 1 and gall index ≤ 2). Two of the resistant progeny (8.7%) were predicted to be susceptible with the marker. There was only one sample which could not be amplified by the marker primers. In the susceptible progenies (egg mass index > 1 , gall index > 2) the marker amplification matched 100% with the phenotype classification. These data show that the marker genotype generated with primer set 197/909 is strongly correlated with the nematode resistance phenotype data. The reproducibility of this marker for selecting nematode resistance has been independently confirmed (Dr. Jim Starr, personal communication, 2005).

The evaluation of results from the new marker on both the segregating population and the advanced breeding lines determined that there was a high correlation between marker and nematode resistance phenotype data. In the segregating population, we observed 3 and 6.3% discrepancies in the $F_{2.3}$ families and F_3 individuals, respectively, where the dominant marker for resistance was not amplified even though the phenotype data suggested that they were resis-

tant. These discrepancies most likely were due to recombination between the marker and resistance gene which was reported to be 5.8% (Burow et al., 1996).

Nematode resistant plant selections made by the evaluation of nematode reproduction is labor intensive and time consuming. Currently, most of the resistant progeny screening has to be performed in the greenhouse due to the requirement of controlled environment for nematode growth. It requires large numbers of nematode eggs for inoculum, several replicated pots (plants) per entry, and 2 mo for nematode reproduction. Moreover, scoring eggs and galls produced on the root system of each plant destroys the plant. The only way to rescue the plant is through cuttings which means another 4 mo of growth period before harvesting. Using marker-assisted selection, it takes only one leaflet from the plant for resistance identification and all the progenies carrying the nematode resistance trait can be saved for seed production. Field screening for resistance also becomes feasible. Without the limitation of space, thousands of seeds from a segregating population can be evaluated in the same growing season.

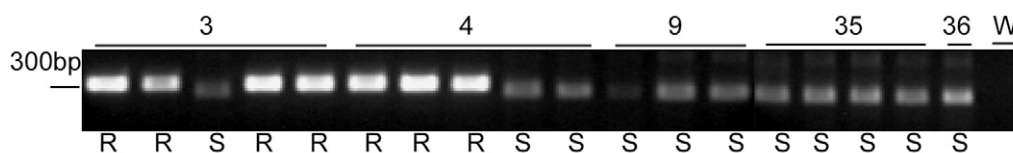


Figure 3. F_3 individuals were amplified by primer set 909/197 to confirm the scoring for the reconstructed F_2 population. Sample numbers correspond to the F_2 parents. Lanes within each sample number are F_3 individuals. Samples are separated on 2% agarose gel. W, water negative control; R, resistant; S, susceptible.

Molecular marker–assisted selection would improve the efficiency of identifying resistant plants and hasten breeding efforts to combine nematode resistance with other important characteristics in peanut.

The new dominant marker 197/909 developed in this study was shown to be a reliable predictor for nematode resistance. Since this marker is PCR based, the screening is fast and effective. We have used both DNeasy and the high-throughput extraction method of Xin et al. (2003) for DNA preparation. The 197/909 marker in combination with the high-throughput DNA extraction method reduced the cost of screening (extraction, labor, and PCR reagents) from ~\$4.50 per plant to <\$0.80 per plant (includes the cost of repeating the assay on 10% of the plants due to the higher failure rate). Usually a peanut breeding program requires screening thousands of plants in a segregating population which would be economically feasible using the high-throughput DNA extraction method. With marker-assisted selection, labor costs for nematode inoculation and counting are transferred to DNA extraction and gel assays.

The goal to derive host–plant resistance to nematodes frequently has required introgression of resistance genes into cultivars through interspecific crosses. Nematode resistant tomato (Seah et al., 2004), carrot (*Daucus carota* L.) (Boiteux et al., 2004), and plum (*Prunus cerasifera* Ehrh.) (Lecouls et al., 2004) as well as peanut are examples of introgression. Since cultivated peanut is relatively monomorphic within the species with regard to DNA sequence (Kochert et al., 1991), marker-assisted selection is likely to be more successful with traits that have been introgressed through interspecific crosses. Selection for nematode resistance has been accelerated in a few crops by marker-assisted selection. For example, discoveries of a marker closely linked to *Mi* in tomato (Williamson et al., 1994) and two SCAR markers linked to *Ma* in plum (Lecouls et al., 2004) have been successfully applied to selection from segregating interspecific progenies. In our study, we have developed an easily scorable PCR-based marker for peanut nematode resistance. The marker demonstrates good correlation with phenotype data. Since this marker amplifies fragments of different sizes from susceptible and resistant plants, the chance for false negative results due to amplification failures can be minimized. Application of this marker in peanut breeding programs would be a powerful tool for pyramiding nematode resistance with other genetic traits. However, since the resistance marker showed 5.8% recombination with the nematode resistance gene, additional markers that are more tightly linked with nematode resistance need to be developed. The presence of moderate nematode resistance in the GP-NC WS14 genotype supports the observation of Holbrook et al. (2003) that other genes may be involved in the inheritance of nematode resistance in peanut lines of interspecific ori-

gin. We are currently trying to develop new nematode resistance markers by amplified fragment length polymorphism and several potential markers have been identified. Genetic mapping and amenability to SCAR conversion will confirm whether any markers in addition to RKN440 will be useful for marker-assisted selection for nematode resistance in peanut.

Acknowledgments

Mrs. Evelyn P. Morgan provided excellent technical assistance for this work. Mr. Benjamin G. Mullinix conducted the statistical analysis.

References

- Anderson, W.F., P. Timper, and C.C. Holbrook. 2006. Registration of root-knot nematode resistant peanut germplasm lines NR 0812 and NR 0817. *Crop Sci.* 46:481–482.
- Baltensperger, D.D., R.A. Dunn, and G.M. Prine. 1986. Root-knot nematode resistance in *Arachis glabrata*. *Peanut Sci.* 13:78–80.
- Boiteux, L.S., J.R. Hyman, I.C. Bach, M.E.N. Fonseca, W.C. Matthews, P.A. Roberts, and P.W. Simon. 2004. Employment of flanking codominant STS markers to estimate allelic substitution effects of a nematode resistance locus in carrot. *Euphytica* 136:37–44.
- Burow, M.D., J.L. Starr, A.H. Paterson, and C.E. Simpson. 1996. Identification of peanut (*Arachis hypogaea* L.) RAPD markers diagnostic of root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood) resistance. *Mol. Breed.* 2:369–379.
- Garcia, G.M., H.T. Stalker, E. Shroeder, 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.
- Holbrook, C.C., A.K. Culbreath, and P. Timper. 2003. Resistance to tomato spotted wilt virus and root-knot nematode in peanut interspecific breeding lines. *Crop Sci.* 43:1109–1113.
- Holbrook, C.C., A.W. Johnson, and M.G. Stephenson. 2000. Level and geographical distribution of resistance to *Meloidogyne arenaria* in the U.S. peanut germplasm collection. *Crop Sci.* 40:1168–1171.
- Holbrook, C.C., and J.P. Noe. 1990. Resistance to *Meloidogyne arenaria* in *Arachis* spp. and the implications on development of resistant peanut cultivars. *Peanut Sci.* 17:35–38.
- Holbrook, C.C., M.G. Stephenson, D.W. Gorbet, and J.P. Noe. 1998. Evaluation of peanut breeding lines with resistance to the peanut root-knot nematode. *Crop Sci.* 38:260–262.
- Hussey, R.S., and K.R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Dis. Rep.* 57:1025–1028.
- Hussey, R.S., and G.J.W. Janssen. 2002. Root-knot nematodes: *Meloidogyne* species CABI, Engham, UK.
- Johnson, A.W., A.K. Culbreath, G.J. Gascho, S.H. Baker, N.A. Minton, T.B. Brenneman, and G.W. Burton. 1999. Bahiagrass, corn, cotton rotations, and pesticides for managing nematodes, diseases, and insects on peanut. *J. Nematol.* 31:191–200.
- Jung, S., K. Moore, A.G. Abbott, G. Kochert, P.L. Tate, and R. Horn. 2003. The phylogenetic relationship of possible progenitors of the cultivated peanut. *J. Hered.* 94:334–340.
- 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.

- Lecouls, A.C., V. Bergougnoux, M.J. Rubio-Cabetas, N. Bosselut, R. Voisin, J.L. Poessel, M. Faurobert, A. Bonnet, G. Salesses, and E. Dirlewanger. 2004. Marker-assisted selection for the wide-spectrum resistance to root-knot nematodes conferred by the Ma gene from Myrobalan plum (*Prunus cerasifera*) in interspecific *Prunus* material. *Mol. Breed.* 13:113–124.
- Minton, N.A., and P. Baujard. 1990. Nematode parasites of peanut. p. 285. In M. Luc (ed.) *Plant parasitic nematodes in subtropical and tropical agriculture*. Vol. 1. CAB International, Wallingford, Oxfordshire, UK.
- Nelson, S.C., C.E. Simpson, and J.L. Starr. 1990. Expression of resistance to *Meloidogyne arenaria* in *Arachis batizocoi* and *A. cardenasii*. *J. Nematol.* 22:242–244.
- Nelson, S.C., J.L. Starr, and C.E. Simpson. 1989. Resistance to *Meloidogyne arenaria* in *Arachis* spp. germplasm. *J. Nematol.* 21:654–660.
- Ramos, M.L., G. Fleming, Y. Chu, Y. Akiyama, M. Gallo, and P. Ozias-Akins. 2006. Chromosomal and phylogenetic context for conglutin genes in *Arachis* based on genomic sequence. *Mol. Genet. Gen.* 275:578–592.
- Rodriguez-Kabana, R., C.F. Weaver, L. Wells, N. Kokalis-Burelle, and D.G. Robertson. 1995. Effects of partridge pea-peanut rotations on populations of *Meloidogyne arenaria*, incidence of *Sclerotium rolfsii*, and yield of peanut. *Nematropica* 25:27–34.
- Seah, S., J. Yaghoobi, M. Rossi, C.A. Gleason, and V.M. Williamson. 2004. The nematode-resistance gene, Mi-1, is associated with an inverted chromosomal segment in susceptible compared to resistant tomato. *Theor. Appl. Genet.* 108:1635–1642.
- Seijo, J.G., D. Ducasse, E.A. Moscone, A. Krapovickas, G.I. Lavia, and A. Fernandez. 2004. Physical mapping of the 5S and 18S–25S rRNA genes by FISH as evidence that *Arachis duranensis* and *A. ipaensis* are the wild diploid progenitors of *A. hypogaea* (*Leguminosae*). *Am. J. Bot.* 91:1294–1303.
- Simpson, C.E. 2001. Use of wild *Arachis* species/introgression of genes into *A. hypogaea* L. *Peanut Sci.* 28:114–116.
- Simpson, C.E., M.D. Burow, A.H. Paterson, J.L. Starr, and G.T. Church. 2003. Registration of ‘NemaTAM’ peanut. *Crop Sci.* 43:1561.
- Simpson, C.E., and J.L. Starr. 2001. Registration of ‘COAN’ peanut. *Crop Sci.* 41:918.
- Stalker, H.T., K.R. Barker, B.B. Shew, and M.K. Beute. 2002a. Registration of two root-knot nematode-resistant peanut germplasm lines. *Crop Sci.* 42:312–313.
- Stalker, H.T., M.K. Beute, B.B. Shew, and T.G. Isleib. 2002b. Registration of five leaf spot-resistant peanut germplasm lines. *Crop Sci.* 42:314–316.
- Starr, J.L., T.A. Lee, Jr., and C.E. Simpson. 1995. Resistance to *Meloidogyne arenaria* in advanced generation breeding lines of peanut. *Peanut Sci.* 22:59–61.
- Williamson, V.M., J.Y. Ho, F.F. Wu, N. Miller, and I. Kaloshian. 1994. A PCR-based marker tightly linked to the nematode resistance gene, Mi in tomato. *Theor. Appl. Genet.* 87:757–763.
- Xin, Z., J.P. Velten, M.J. Oliver, and J.J. Burke. 2003. High-throughput DNA extraction method suitable for PCR. *Bio-techniques* 34:820–826.