Resistance to *Meloidogyne arenaria* in Advanced Generation Breeding Lines of Peanut[1]

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ABSTRACT

Levels of resistance to the root-knot nematode *Meloidogyne arenaria* in F_2 individuals from the second, third, and fourth backcross (BC) generations were compared in seven separate tests to that of the root-knot nematode-resistant peanut germplasm line TxAG-7. Resistance of TxAG-7 was derived from the wild species *Arachis batizocoi*, *A. cardenasii*, and *A. diogoi*. Recurrent susceptible parents were Florunner and Tamnut 74 for the all backcrosses, Tamspan 90 for BC_3 and BC_4, and NC 7 and VC-1 for BC_4. Resistance in these tests was defined as an inhibition of nematode reproduction relative to that of the susceptible recurrent parent. Numerous individuals with a level of resistance similar to that of TxAG-7 were identified from each backcross generation. In three field tests, the resistant BC_2 genotype TP-223 supported a lower final nematode population density than did its susceptible recurrent parent Florunner. When rooted cuttings from selected BC_4F_2 individuals were retested to confirm the original resistance class, ratings were unchanged for those originally identified as resistant or susceptible. Of nine individuals originally identified as having moderate resistance (2.5 to 12.5% of the eggs/g roots as the susceptible recurrent parent), one was identified as susceptible, one as moderately resistant, and seven as resistant (<2.5% of the eggs/g roots) upon retest. These data are evidence that this source of resistance is readily recoverable from advanced backcross generations.

Key Words: Groundnut, host resistance, peanut, plant breeding, root-knot nematode, *Arachis* spp.

Root-knot nematodes, especially *Meloidogyne arenaria* (Neal) Chitwood, are important pathogens of peanut throughout the southern United States. These nematodes infest as much as 40% of the peanut production fields in some states (5,6); approximately 30% of the fields in Texas are infested (14). Yield losses caused by root-knot nematodes can be reduced by several management tactics, including use of crop rotation (9) and nematicides (10). Unfortunately, no peanut cultivar with resistance to *M. arenaria* is available to peanut producers. Several potentially useful sources of resistance, however, have been identified (3,7,11)

We have described resistance to *M. arenaria* in several wild *Arachis* spp. germplasm lines (7) and have developed nematode-resistant genotypes, TxAG-6 and TxAG-7 (formerly TP-129 and TP-135-4, respectively), which are cross-compatible with *A. hypogaea* (12). These two germplasm lines were derived from the three wild species, *A. batizocoi* Krapov. and W. C. Gregory (K 9484), *A. cardenasii* Krapov. and W. C. Gregory (GKP 10017), and *A. diogoi* Hoehne (GKP 10602) (formerly *A. chacoensis*). TxAG-6 is the F_1 of the cross 4x[*A. batizocoi* x (*A. cardenasii* x *A. diogoi*)]. TxAG-7 is from the first backcross of the cultivar Florunner x TxAG-6 with Florunner as the recurrent parent. The resistance mechanism of *A. batizocoi* differs from that of *A. cardenasii* with the former being expressed as a reduction in numbers of invading juveniles that are able to complete their life cycle and an increase in the generation time (8). Resistance in *A. cardenasii* is expressed as a complete inhibition of development of invading juveniles and may be a hypersensitive reaction (10). Preliminary data suggest that the resistance of *A. cardenasii* is conditioned by a few major dominant genes (13).

TxAG-7 has been used as a source of resistance in a backcrossing introgression program to develop advanced generation breeding lines with high levels of resistance to *M. arenaria*. This report documents the progress that has been achieved in that program.

Materials and Methods

Resistance to *M. arenaria* in F_2 individuals derived from the second, third, and fourth backcrosses (BC_2, BC_3, and BC_4, respectively) was assessed in greenhouse tests. For each backcross generation, stem cuttings were collected from nematode-resistant F_2 individuals from the previous backcross generation plants and rooted in peat. Pollen collected from the rooted, nematode-resistant cuttings was used to pollinate recurrent parents and to achieve the next backcross generation. Florunner and Tamnut 74 were used as recurrent parents for all backcross generations, whereas Tamspan 90 was used for BC_3 and BC_4, and NC 7 and VC-1 were used for BC_4. Ten seeds from each F_2 genotype to be tested were planted separately into 15-cm diameter pots filled with a coarse sand-peat soil mix (6:1, v/v). Additional pots were planted to the recurrent susceptible parent or to the nematode-resistant TxAG-7. Individual plants were inoculated with a suspension of 10,000 eggs of *M. arenaria* race 1 isolates #82-4 or #92-26. These isolates of *M. arenaria* were obtained from peanut in Texas and stock cultures were maintained on *Lycopersicon esculentum* Mill. cv. Rutgers. Inoculum was prepared by extracting nematode eggs from infected tomato roots with 0.052% NaClO (4). Species identification of the nematode isolates was confirmed by esterase phenotype (2).

Inoculated plants were maintained in a greenhouse at 24-30 C after inoculation and were fertilized once with a slow release formulation of N-P-K. Insect and mite pests were controlled with periodic applications of nonsystemic insecticides. Plants were harvested 8 wk after inoculation, the soil washed from the roots with tap water, the roots were

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blotted dry, and then weighed. Nematode eggs were extracted from whole root systems with 1.04% NaClO (4) and counted using a stereo microscope.

Individual plants were categorized as resistant if they had <2.5% of the numbers of eggs/g of fresh root weight as did the susceptible recurrent parent, moderately resistant if the numbers of eggs were 2.5 to 12.5% of the recurrent parent, or susceptible if the numbers of eggs produced were >12.5% of the susceptible recurrent parent (7). To confirm the resistance category for individuals in the BC_4F_2 generation, cuttings of selected genotypes were rooted in peat. Cuttings with well developed adventious roots were then transplanted into 15-cm diameter pots and inoculated with *M. arenaria* as described above. Nematode reproduction on these cuttings was measured as eggs/g roots at 8 wk after inoculation. Florunner planted from seed was used as the susceptible control.

In addition to the greenhouse tests, reproduction of *M. arenaria* on one genotype (TP-223) from the second backcross generation was compared to reproduction on its susceptible recurrent parent (Florunner) in three field tests. The 1992 test was in Comanche County, TX where the two genotypes were each planted in single row plots, 12 m long, with 10 replications. Two tests were conducted in 1993, one each in Comanche and Erath counties. These plots had two rows, 24 m long and, were replicated nine times. The seed of TP-223 for the 1992 test were BC_2F_3, whereas seed for the 1993 tests were BC_2F_4. For each test, initial and final nematode population densities were estimated from composite soil samples collected from each plot. Eight to 12 soil cores (2.5-cm diameter x 25-cm deep) were collected from the root zone of each plot for each composite sample. A subsample of 500 cm^3 of soil from each sample was processed by elutriation and centrifugation (1) to determine the numbers of juvenile nematodes and eggs that were present.

Results

A total of 554 individuals from all backcross generations were examined for resistance to *M. arenaria* in seven separate tests. Reproduction of *M. arenaria* on the different susceptible recurrent parents was variable across all tests, ranging from 820 eggs/g roots for NC 7 in the BC_4F_2 test to 9880 eggs/g roots on Tamnut 74 in the same test. In each test, reproduction of *M. arenaria* on TxAG-7 was lower ($P \leq 0.01$) than on any of the susceptible recurrent parents, ranging from 0 to 70 eggs/g roots. Numbers of individuals resistant to *M. arenaria* identified in each backcross generation ranged from 24.7 to 74.5% of those tested (Table 1). Susceptible individuals ranged from 14.7 to 54.9% of those tested. Except for the BC_2F_2, with Florunner as the recurrent parent, the moderately resistant category always contained fewer individuals than did the resistant or susceptible categories. No other obvious trend with respect to segregation of resistance was noted from these data.

When rooted cuttings were collected from selected BC_4F_2 individuals and retested for resistance to *M. arenaria*, all 12 resistant genotypes were confirmed as resistant and the five susceptible genotypes were confirmed as susceptible (Table 2). *Meloidogyne arenaria* produced fewer eggs/g root ($P=0.05$) on the resistant genotypes than on Florunner. Of the nine genotypes

Table 1. Resistance to *Meloidogyne arenaria* among F_2 individuals from the second, third, and fourth backcross generations.[a]

Recurrent parent	N	Resistant	Moderately resistant	Susceptible
			%	
			BC_2F_2	
Florunner	81	24.7	33.3	42.0
Tamnut 74	61	42.6	19.7	37.7
			BC_3F_2	
Florunner	102	74.5	10.8	14.7
Spanish[b]	40	55.0	7.5	37.5
			BC_4F_2	
Florunner	139	34.5	13.7	51.8
Spanish[b]	91	27.5	17.6	54.9
Virginia[c]	40	55.0	5.0	40.0

[a]Resistance is defined based on nematode reproduction (eggs/g roots) with resistance <2.5% of reproduction on the susceptible recurrent parent, moderately resistant = 2.5 to 12.5% of the susceptible recurrent parent, and susceptible >12.5% of the susceptible recurrent parent.

[b]Spanish market-type recurrent parents were Tamnut 74 and Tamspan 90.

[c]Virginia market-type recurrent parents were NC 7 and VC-1.

Table 2. Resistance to *Meloidogyne arenaria* of rooted cuttings from BC_4F_2 individuals previously determined to differ in resistance to *M. arenaria*.

Genotype[a]	No. tested	Initial rating[2]	Eggs/g roots[c] Mean	Eggs/g roots[c] Highest	Second rating[b]
			no.		
Florunner	4	S	6500	9300	S
TP-245-6A-6	4	S	2700	9670	S
TP-247-1A-3	5	R	20	50	R
TP-247-2A-1	4	MR	550	1100	MR
TP-248-4A-6	6	R	0	0	R
TP-249-5B-1	6	R	50	120	R
TP-249-5B-2	4	MR	160	620	R[d]
TP-249-5B-9	5	R	50	190	R
TP-250-2A-1	6	MR	5470	7700	S[d]
TP-250-2A-8	5	S	10110	18030	S
TP-251-3B-2	4	R	100	330	R
TP-251-3B-7	4	S	3470	7160	S
TP-252-1A-5	3	R	30	90	R
TP-252-2A-3	6	R	0	0	R
TP-252-2A-10	3	S	2960	4680	S
TP-252-4A-1	5	S	5870	9260	S
TP-252-4A-5	6	R	10	10	R
TP-252-4A-6	5	MR	20	100	R[d]
TP-252-5A-3	4	MR	10	10	R[d]
TP-253-4C-4	8	MR	10	70	R[d]
TP-253-4C-8	4	R	140	510	R
TP-253-4C-10	4	MR	10	20	R[d]
TP-254-1A-3	6	MR	10	20	R[d]
TP-254-1A-5	6	R	10	10	R
TP-254-2B-1	4	MR	10	10	R[d]
TP-254-2B-7	7	R	0	0	R
TP-254-3B-2	5	R	10	10	R
$LSD_{0.05}$			3460		

[a]Genotypes TP-245 through TP-252 are runner in market type with Florunner as the recurrent parent. Genotypes TP-253 and TP-254 are spanish in market type, with Tamnut 74 and Tamspan 90, respectively, as recurrent parents.

[b]R = resistant with <2.5% of nematode reproduction of susceptible parent; MR = moderately resistant with 2.5 to 12.5% of susceptible parent; S = susceptible with >12.5% of nematode reproduction of susceptible parent.

[c]Data from second test for resistance and used to determine second rating.

[d]Denotes genotypes for which resistance rating changed upon retest.

initially identified as moderately resistant, one was identified as susceptible and seven as resistant in the retest.

In the three field tests, initial nematode population densities were less than 50 eggs and juveniles/500 cm^3 of soil and did not differ between treatments. The *M. arenaria*-resistant genotype TP-223 from the second backcross generation supported lower ($P \leq 0.05$) final nematode populations in all field tests than did its susceptible recurrent parent Florunner. Mean final nematode population densities on TP-223 ranged from 9.5 to 16.6% of those on Florunner (Fig. 1). In each test, one replication of the TP-223 had a nematode population density that was similar to that of the mean of the Florunner plots.

Discussion

Relatively large numbers of individuals with a high level of resistance to *M. arenaria* were recovered from each backcross generation, regardless of whether the susceptible recurrent parent was a runner, spanish, or virginia market-type peanut. We have not detected a reduction in the level of resistance during the backcrossing program. Additionally, the data from the three field studies suggest that the resistance of these genotypes will provide a high level of resistance to the nematode throughout the growing season in naturally infested fields. The occurrence of a few susceptible individuals in the BC_2F_3 and BC_2F_4 was not unexpected since there was no selection to ensure that only resistant individuals were used for seed increase in that study. With a rigorous selection program, the susceptible individuals will be easily removed from the population. If the susceptible individuals had not been present in these populations, then final population densities on TP-223 in the remaining plots would have ranged from 1.5 to 5.5% of the densities on Florunner.

The accuracy with which resistant and susceptible individuals were identified in the BC_4F_2 population has validated our selection procedure because only individuals identified as resistant have been used as male parents in our backcrossing program. That most of the individuals initially identified as moderately resistant were found to be either resistant or susceptible in the retest of the rooted cuttings indicates that our moderate resistance category is an artifact due to experimental error. The lack of moderate or partial resistance in this F_2 population would suggest that resistance is conditioned by a few major affect genes. Preliminary genetic analysis of *A. cardenasii* also indicated that resistance of this species is governed by a few major genes (13).

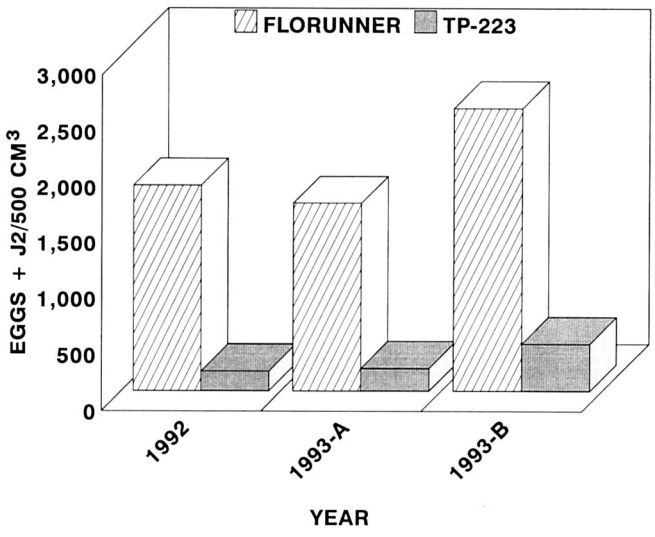

Fig. 1. Comparison of final population densities of *Meloidogyne arenaria* on susceptible Florunner and resistant TP-223 in field plots. Differences between Florunner and TP-223 for each test were significant at P=0.05.

Little effort beyond subjective analysis of plant and pod appearance has been spent on evaluation of agronomic characters of each backcross generation. An increase in seed size (data not shown), however, has been observed with each backcross generation. We believe that it will be possible to develop agronomically superior peanut genotypes with high levels of resistance to *M. arenaria* from these nematode-resistant breeding lines. Additionally, these data are evidence of that genetic resources present in the wild *Arachis* species germplasm can be used to improve cultivated peanut.

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