Resistance to *Meloidogyne arenaria* in *Arachis* spp. Germplasm¹

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Abstract: Field and greenhouse evaluations of 116 wild Arachis spp. genotypes demonstrated the presence of resistance to reproduction of the root-knot nematode Meloidogyne arenaria race 1. Resistance in greenhouse tests was based on test lines having ≤ 2.5% of the number of eggs per gram of roots as did the susceptible A. hypogaea cv. Tamnut 74. In field tests, resistant genotypes were identified on the basis of having lower (P = 0.05) final nematode population densities than did Tamnut 74. Resistance was identified in genotypes from 11 of 15 wild species tested and in 10 of 20 genotypes belonging to undescribed species. Results of field and greenhouse experiments were similar; 26 of 31 genotypes common to both tests gave similar responses in both tests. Resistance to M. arenaria was identified in the complex hybrid TP-135, which was derived from A. hypogaea cv. Florunner × (A. batizocoi K 9484 × [A. cardenasii GKP 10017 × A. chacoensis GKP 10602])4×. In a single greenhouse test, three of six genotypes resistant to M. arenaria were also resistant to M. hapla. These data indicate that the Arachis spp. germplasm contains several sources of resistance to M. arenaria and possibly M. hapla. Some of this resistance is in germplasm that is genetically compatible with A. hypogaea. The complex hybrid TP-135 incorporates resistance from wild species into the genetic background of A. hypogaea. On the basis of these data, we believe it may be possible to develop peanut cultivars with high levels of resistance to M. arenaria and M. hapla.

Key words: Arachis hypogaea, Arachis spp., Meloidogyne arenaria, M. hapla, peanut, resistance.

Peanut, Arachis hypogaea L., is a host for the root-knot nematodes Meloidogyne arenaria race 1 (Neal) Chitwood and M. hapla Chitwood (15). These nematodes cause substantial peanut yield losses in the United States (17,18). Traditionally, management practices for M. arenaria have relied upon crop rotations and nematicides (15). Since peanut cultivars resistant to, or tolerant of, M. arenaria or M. hapla are not available, the development of resistant cultivars is desirable.

Previous searches within Arachis spp. germplasm for genotypes resistant to Meloidogyne spp. have been intensive, yet primarily limited to A. hypogaea. None of the more than 2,700 A. hypogaea genotypes tested exhibited high levels of resistance to M. arenaria (7,11,12). Of 371 genotypes evaluated for resistance to M. hapla, low levels of resistance were reported in some (2). Resistance to M. arenaria and M. hapla has been reported within other Arachis spp.

Resistance to M. hapla was found in other Arachis spp. but they are genetically incompatible with cultivated peanut (2). In another study, 235 cultivars, breeding lines, and plant introductions of A. hypogaea and 12 Arachis spp. genotypes were tested for resistance to M. hapla; eight of the peanut cultivars were classified as moderately susceptible, four Arachis spp. were resistant, and all other genotypes were highly susceptible (6). The interactions of A. glabrata with root-knot nematodes were studied and high levels of resistance to M. arenaria were reported (1), but the tetraploid A. glabrata is not cross-compatible with A. hypogaea (Simpson, unpubl.).

The objectives of this study were 1) to evaluate the wild *Arachis* spp. collection of the Texas Agricultural Experiment Station for resistance to *M. arenaria* and 2) to evaluate selected genotypes for their reaction to *M. hapla*.

MATERIALS AND METHODS

Greenhouse evaluation: One hundred sixteen Arachis spp. and two complex hybrids were compared with the susceptible A. hypogaea cv. Tamnut 74 for ability to support M. arenaria reproduction in 17 separate greenhouse tests. The complex hybrid TP-

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129 is the F₁ of the cross (A. batizocoi K 9484 \times [A. cardenasii GKP 10017 \times A. chacoensis GKP 10602])4×. TP-135 is the first backcross generation from A. hypogaea cv. Florunner × TP-129 with Florunner as the recurrent parent. Ten seeds of each entry were dusted with the fungicide thiram, placed into moist, rolled germination paper, and incubated at 25-28 C. Because of seed dormancy in some species, seeds of all genotypes were treated with 0.01 M ethrel, pH 6.0, to break their dormancy (10). After 4 days, seedlings were transplanted singly into 15-cm-d pots (1,240 cm³) containing a 4:1 (v:v) mixture of pasteurized sand and peat and arranged in a completely randomized design on the greenhouse bench. Soil temperatures ranged from 25 to 35 C.

Eggs of M. arenaria race 1, originally collected from infected peanut, were extracted from 8-12-week-old cultures maintained on tomato (Lycopersicon esculentum Mill. cv. Rutgers) (8). Each Arachis spp. seedling was inoculated with 5,000 nematode eggs distributed into four depressions equidistant from the base of 2-3-week-old plants. Eight weeks after inoculation, plants were harvested and the roots were rinsed with tap water. Roots were blotted dry and weighed, and eggs were extracted by treatment with 0.5% NaOCl (8).

Nematode reproduction, measured as eggs per gram of fresh root tissue, was the criterion upon which assessments of resistance were based. The ratio of nematode reproduction on test lines to reproduction on Tamnut 74 was expressed as a percentage. Resistance categories were established as follows: resistant = $\leq 2.5\%$ of Tamnut 74, moderately resistant = 2.6– 12.5%, moderately susceptible = 12.6-62.5%, and susceptible = > 62.5% of Tamnut 74. The reproduction factor (RF) was defined as the ratio of final M. arenaria population density to the initial population density of 5,000 eggs (14) and was calculated for each germplasm line. Data were subjected to analysis of variance by the SAS (16) general linear models procedure.

Six Arachis spp. genotypes, the two complex hybrids, and the A. hypogaea cultivars

Florunner and Tamnut 74 were similarly tested to determine their response to M. hapla. The procedures used in this single test were identical to those used to test for resistance to M. arenaria. The M. habla isolate, provided by K. R. Barker of North Carolina State University, was obtained from infected peanut in North Carolina and maintained on tomato.

Field evaluation: Two field experiments were conducted in 1987 at the Texas A&M University Plant Disease Research Station at Yoakum, Texas, to obtain comparisons of field and greenhouse performance of selected lines. Both experiments were conducted in a field artificially infested with M. arenaria race 1 in 1985 and planted to peanut every year thereafter. The soil type was a loamy sand (83.6% sand, 5% silt, 11.4% clay; pH 6.9; < 1% organic matter). Field test 1 consisted of 10 treatments (nine Arachis genotypes and Tamnut 74) in a randomized complete block design with five replications. Plots were single rows, 1.2 m long on 0.9-m centers. Because genotypes of A. glabrata do not readily produce seed, they were planted as rooted cuttings. Five plants were established in each plot. Field test 2 consisted of 23 treatments (22 Arachis spp. genotypes and Tamnut 74) in a randomized complete block design with four replications. Plots were single rows, 3.1 m long on 0.9-m centers. Ten plants were established in each plot. Seed of all entries in both tests were allowed to germinate in moisture chambers for 72 hours at 28 C and, because light inhibits hypocotyl elongation of many Arachis spp. (Simpson, unpubl.), they were planted in darkness to enhance emergence. Fewer plants were used in field test 1 than test 2 because of limited seed availability.

Meloidogyne arenaria population densities were determined before planting, 8 weeks after planting, and 1 week before harvest. Composite samples of 10 soil cores, each $2.5 \text{ cm d} \times 20 \text{ cm deep, were collected}$ from each plot (3,5). Subsamples of 500 cm³ soil were processed by elutriation (4). Juveniles (J2) were recovered from the elutriated samples via centrifugation (9), and

Table 1. Arachis spp. genotypes identified as resistant to Meloidogyne arenaria race 1 in greenhouse tests.

Genotype		Plant introduction	Percentage of		
	Collector ID†	number (PI)	Eggs/g root	Tamnut 74‡	Host statu
A. chacoensis	GKP 10602	276235	73	1	R
A. cardenasii	GKP 10017	262141	0	0	R
A. batizocoi	GKPBSSc 30083	468329	10	1	R
A. batizocoi	K 9484	298639	23	1	R
A. villosa			0	0	R
A. villosa	S 862-1		30	1	R
A. villosa	S 863-1		0	0	R
A. villosa	S 865-1		0	1	R
A. stenosperma	HLK 410	338280	3	1	R
A. stenosperma	VSGeMoSv 7379	497579	0	0	R
A. stenosperma	VSGeMoSv 7377	497578	0	0	R
A. stenosperma	VSGeMoSv 7384	497581	4	1	R
A. duranensis	GKPSSc 30071	475846	1,723	10	MR
A. duranensis	GKPBSSc 30078	468324	45	1	R
A. duranensis	KSBScC 36003	475883	346	2	R
Arachis sp.	AViW 2796	497546	4	2	R
Arachis sp.	VSGeStW 7762		0	0	R
Arachis sp.	VKRSv 7639-1		30	1	R
Arachis sp.	V9470-1		175	5	MR
Arachis sp.	CIAT 9660		3	1	R
Arachis sp.	VSGr 6396		5	1	R
Arachis sp. Arachis sp.	VSGdStW 7764yf		5	1	R
Arachis sp.	VPoBi 9146		170	2	R
Arachis sp. Arachis sp.	VSW 9923		30	ī	R
Arachis sp. Arachis sp.	VSGr 6407-1		1	ī	R
Complex hybrid	TP-129		î	i	R
Complex hybrid	TP-135		2	ì	R
A. glabrata	GKP 9591	262827	88	î	R
A. glabrata A. glabrata	GKP 9797	262807	15	î	R
A. glabrata A. glabrata	GKP 9830	262797	0	ō	R
	GKP 9645	262841	1	ĭ	R
A. glabrata	GKP 9918	262294	0	0	R
A. glabrata	GKP 9567	262814	ő	0	R
A. glabrata	GKP 9649	262844	1	1	R
A. glabrata	GKP 9049 GKP10120	276202	4	1	R
A. glabrata			1	1	R
A. glabrata	GKPSc 30132	468175		1	R
A. glabrata	GK 30021	468161	1	0	R
A. glabrata	A 43	231318	0		
A. glabrata	GKP 10596	276233	1	1	R R
A. sylvestris	VSW 6676	497567	0	0	
A. sylvestris	VSW 6785	497545	46	1	R
A. sylvestris	VVeSv 6180		25	1	R
A. sylvestris	VKRSv 6575		5	1	n
A. sylvestris	VVeSv 8373		167	2	R
Arachis sp.	VKVeSv 8458		0	0	R
Arachis sp.	VRGeSv 7560		8	1	R
Arachis sp.	VRGeSv 7644-1	10== 20	0	0	R
Arachis sp.	VSW 6709	497568	45	1	R
A. pinto	GKP 12787	338447	21	1	R
A. paraguariensis	GKP 10585	276231	275	2	R
A. paraguariensis	VRGeSv 7644-1		0	0	R
A. paraguariensis	GKP 9646	262842	0	0	R
Arachis sp.	GK 30013	468155	1,037	10	MR
Arachis sp.	VSGr 6340-2	476105	148	2	R
Arachis sp.	VSGr 6340	476105	73	2	R
Arachis sp.	VeSv 6001	476135	0	0	R
A. macedoi	GKP 10127	276203	787	8	MR

[†] Collector's initials as follows: A = Allem, B = Banks, Bi = Bianchetti, C = Coradin, Ge = Gerin, Go = Godoy, G = Gregory, Gr = Gripp, H = Hammons, He = Hemsy, K = Krapovickas, L = Langford, Mo = Moss, P = Pietraelli, Po = Pott,

		Percentage of _ Tamnut 74†	Host status‡	
Genotypes	Eggs/g root		M. hapla	M. arenaria§
A. duranensis GKPSSc 36006	6,737	234.0	S	s
A. hypogaea Tamnut 74	2,879	100.0	S	S
A. duranensis GKPBSSc 30069	2,564	89.1	S	S
A. hypogaea Florunner	2,135	74.2	S	S
A. batizocoi K 9484	777	27.0	MS	R
Complex hybrid TP-135∥	406	14.1	MS	R
A. chacoensis GKP 10602	250	8.7	MR	R
A. cardenasii GKP 10017	61	2.1	R	R
Complex hybrid TP-129	60	2.1	R	R
A. stenosperma HLK 410	0	0.0	R	R

§ Host status with respect to M. arenaria based on data from other tests, see Tables 1 and 3.

the egg fraction was recovered after treatment of the root debris with NaOCl (8). Population counts were transformed to log (x + 1) to stabilize variances, and transformed data were subjected to analysis of variance.

RESULTS

Greenhouse evaluation: Although at least 10 seeds of each entry were available, poor germination and seedling diseases resulted in fewer surviving plants for some entries. All data are from a minimum of three replicate plants per entry.

A significant genotype effect $(P \le 0.01)$ upon eggs per gram of fresh root and RF was observed in 16 of 17 experiments. Tamnut 74 averaged 7,440 eggs/g root across the 17 individual experiments. Resistance to M. arenaria was identified in genotypes of 11 of the 15 species tested and in 10 of the 20 genotypes tested which belong to as yet undescribed species. Of the 116 Arachis spp. genotypes and two complex hybrids evaluated, 53 genotypes were resistant and 4 were moderately resistant

(Table 1). Data from genotypes rated as susceptible are not presented. Complex hybrids TP-129 and TP-135 were resistant. RF values were highly variable. Genotypes rated as susceptible, based on percentage of Tamnut 74, had a mean RF of 10.5 (range 0.8 to 47.3); mean RF for resistant genotypes was 0.02 (range 0.0 to 0.23).

Tamnut 74 supported 2,879 eggs/g root in the greenhouse evaluation for resistance to M. hapla. Of the six Arachis spp. genotypes evaluated in this test, two were resistant to M. hapla and one was classified as moderately resistant (Table 2). The complex hybrid TP-129 was rated resistant and TP-135 was moderately susceptible to M. hapla (Table 2).

Field experiment: Initial and midseason population densities in both experiments were highly variable, and no effect of genotype on population densities was detected. Analysis of variance revealed a significant ($P \le 0.05$) genotype effect upon the final population densities of M. arenaria in field tests 1 and 2 (Table 3). Mean separation of the transformed data revealed that 12 genotypes supported lower nematode

[†] Based on eggs per gram of root. ‡ R = resistant, $\leq 2.5\%$ of Tamnut 74; MR = moderately resistant, 2.6-12.5%; MS = moderately susceptible, 12.6-62.5%; S = susceptible, > 62.5% of Tamnut 74.

TP-129 is derived from (A. batizocoi × [A. cardenasii × A. chacoensis])**; TP-135 is the first backcross generation from A. hypogaea cv. Florunner × TP-129 with Florunner as the recurrent parent.

R = Rao, Sc = Silva, S = Simpson, St = Stalker, V = Valls, Ve = Viega, Vi = Vierira, W = Werneck.

[‡] Based on eggs per gram of fresh root.

[§] R = resistant, ≤ 2.5% of Tamnut 74; MR = moderately resistant, 2.6-12.5% of Tamnut 74.

TP-129 is derived from A. batizocoi × (A. cardenasii × A. chacoensis)4×; TP-135 is the first backcross generation from A. hypogaea cv. Florunner × TP-129 with Florunner as the recurrent parent.

TABLE 3. Final population densities of *Meloidogyne arenaria* race 1 on selected *Arachis* spp. genotypes in two field tests.

Genotype	Cultivar or collection number	Final populations†	Host status in greenhouse tests:
-	Test 1		
A. hypogaea	Tamnut 74	1,040	S
Arachis sp.	30013	1,115	MR
A. glabrata	HLKHJe 569	96*	R
$A.\ glabrata$	GKP 9645	66*	R
A. glabrata	GKP 10120	20*	R
A. paraguariensis	GKP 30126	38*	MS
A. cardenasii	GKP 10017	21*	R
A. chacoensis	GKP 10602	6*	R
A. batizocoi	SRS-89/75	4*	MS
Arachis sp.	VeSv 6001	0*	R
	Test 2		
A. hypogaea	Tamnut 74	2,500	S
Arachis sp.	GK 30011	4,230	S
Arachis sp.	GKPSSc 35005	3,840	S
Arachis sp.	GKSSc 30092	2,260	S
Arachis sp.	GK 30008	45*	R
Arachis sp.	VSGeStW 7762	25*	R
Arachis sp.	CIAT 9660	13*	R
Arachis sp.	GKSSc 30097	980	S
A. duranensis	GKPBSSc 30064	1,110	S
A. duranensis	GKPBSSc 30065	1,120	S
A. duranensis	GKPBSSc 30068	200	S
A. duranensis	GKPBSSc 30069	1,020	S
A. duranensis	GKPBSSc 30075	210	MS
A. duranensis	GKPBSSc 30077	700	· S
A. duranensis	GKPBSSc 30078	40*	R
A. duranensis	GKP 10038 11	660	S
A. batizocoi	GKPBSSc 30083	340	R
A. batizocoi	K 9484	220	R
A. rigonii	GKP 10034	300	MS
A. ipaensis	GKPBSSc 30076	2,200	S
A. monticola	GKPBSSc 30062	1,010	S
A. stenosperma	HLK 410	240	R
A. sylvestris	VSW 6676	100	R

^{*} Significantly less than Tamnut 74 ($P \le 0.05$), based on transformed data, log (× + 1), (LSD).

population densities than did Tamnut 74 in these tests.

Pearson's correlation analysis (16) showed a positive correlation (r = 0.59, $P \le 0.01$) between host status of genotypes evaluated in the greenhouse (percentage of Tamnut) and their host status in the field experiments (log [x + 1]). Eleven of fifteen lines rated as resistant or moderately resistant in the greenhouse supported lower ($P \le 0.05$) final population densities of M. arenaria in the field experiments than did Tamnut 74, and only 1 of 16 lines rated

as susceptible or moderately susceptible in greenhouse experiments supported a lower ($P \le 0.05$) final population density in the field experiments.

Discussion

The data presented are evidence of widespread resistance to *M. arenaria* within the genus *Arachis*. Some of this resistant germplasm is genetically compatible with *A. hypogaea*. The resistance to *M. arenaria* among genotypes of *A. glabrata* confirms prior reports (1,6).

[†] Final population densities of M. arenaria eggs and juveniles/500 cm³ soil 1 week before harvest.

[‡] R = resistant, ≤ 2.5% of the number of eggs per gram of root as Tamnut 74; MR = moderately resistant, 2.6-12.5%; MS = moderately susceptible, 12.6-62.5%; S = susceptible, > 62.5% of Tamnut 74.

Because nematode reproduction was variable between experiments, absolute number of nematodes alone was an inadequate measure of resistance. Each wild species germplasm line was, therefore, evaluated as a percentage of Tamnut 74 using the eggs per gram of root data so that between-experiment rankings could be established. RF values ≤ 1.0 generally are indicative of host resistance, but RF values were not as effective as eggs per gram of roots in identifying resistance in these studies because of large differences in root size among genotypes (data not shown). Genotypes with relatively small root biomass can give low RF values because of limited host resources available to support nematode reproduction, yet the same genotype could be equivalent to Tamnut 74 on the basis of eggs per gram of root. Examples of this phenomenon were A. duranensis (GKP 30064) and A. paraguariensis (GKPSc 30126) (13).

Although the primary emphasis of this study was to evaluate wild Arachis spp. germplasm for resistance to M. arenaria, we also examined the response of arbitrarily selected Arachis spp. genotypes to M. hapla. Interestingly, three genotypes resistant to M. arenaria were also resistant to M. hapla. That two genotypes resistant to M. arenaria were moderately susceptible M. hapla suggests that the genes for resistance to the two nematode species differ.

The complex hybrids TP-129 and TP-135 were developed to incorporate resistance to foliar fungal pathogens from wild species into the genetic background of A. hypogaea. That the lines of A. batizocoi, A. cardenasii, and A. chacoensis, used to develop the original complex hybrid from which TP-129 and TP-135 were derived, are also resistant to M. arenaria and M. hapla is fortuitous. Although none of the more than 30 advance generation lines derived from TP-135 which have been evaluated are resistant to M. arenaria (unpubl.), the resistance of TP-135 does prove that the resistance in the wild species germplasm can be moved into the genetic background of A. hypogaea.

In summary, these studies show that the wild Arachis spp. germplasm pool is a source of resistance to M. arenaria and probably M. hapla. Further, this resistance can be incorporated into A. hypogaea by means of complex hybrids. Additional work is needed to determine the number of different sources of resistance that exist, the types of resistance mechanisms that are operative, and the genetic basis for each resistance mechanism.

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