Characterization of *Meloidogyne arenaria*, *M. javanica*, and *M. incognita* reaction of the USA *Arachis pintoi* (Krapov. & W.C. Gregory) germplasm collection

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ABSTRACT

Arachis pintoi Krapov. & W.C. Gregory is a herbaceous, perennial legume, exclusively native to Brazil. It is considered a multiple use legume, being grown for forage; ground cover in fruits orchards, forest, and low tillage systems; erosion control; and ornamental purposes. Accessions of the *A. pintoi* USA germplasm collection of the National Plant Germplasm System (NPGS) were evaluated to characterize its reaction to *Meloidogyne arenaria* (Neal) Chitwood, *M. javanica* (Treub) Chitwood, and *M. incognita* (Kofoid and White) Chitwood. *Arachis pintoi* germplasm presented great variability and high levels of resistance to *M. arenaria*, *M. javanica*, and *M. incognita*.

Key Words: Nematode resistance, tropical legume, genetic resources.

Arachis pintoi Krapov. & W.C. Gregory is a herbaceous, perennial legume, exclusively native to Brazil. It is considered a multiple use legume, being grown for forage; ground cover in fruits orchards, forest, and low tillage systems; erosion control; and ornamental purposes. Although several cultivars have been released in different countries, little is known about the genetic diversity of the germplasm stored at genebanks.

Germplasm characterization consists of studies of eco-geographic and demographic adaptation (Martins, 1984), and according to Solbrig (1980) involves mostly the parameters of the vital cycle of the organism, genetic and physiological studies, plant pathology, and yield evaluation, among other studies. Characterization often also involves taxonomic confirmation and should produce an easy and quick way to differentiate the germplasm, using highly heritable and visible traits (Hawkes *et al.*, 2000). Breeding programs should begin only after appropriate germplasm characterization (Cameron, 1983).

Susceptibility to root-knot nematodes, especially *M. arenaria*, is one of the major problems that groundnut cultivars face in the southeastern USA. *A. pintoi* is considered by some authors as representative of the tertiary or quaternary gene pool of *A. hypogea* L., and so the germplasm could be a source of resistance to *M. arenaria* if genetic barriers could be overcome.

The goal of this research was to evaluate the nematode resistance of several *A. pintoi* germplasm accessions stored at the USDA-NPGS germplasm bank.

Material and Methods

Accessions of *A. pintoi* stored in the Southern Regional Plant Introduction Station of the National Plant Germplasm System (NPGS) located at Griffin, Georgia were transferred to the University of Florida in 2001 and 2002. A list of these accessions with information related to the respective PI numbers and sites of collection is presented in Table 1.

Stems of the germplasm accessions were cut and placed in vermiculate trays under an automated mist system (10 sec every 30 min) for rooting. After 45 d under the mist systems, the rooted cuttings were transferred to 150 cm^3 Conetainers® filled with methyl-bromide-fumigated fine sand topsoil. After transferring, plants were allowed to establish for 2 wk and then used in this experiment. On November 12th of 2003, plants were inoculated with either *M. arenaria* race 1, *M. javanica*, or *M. incognita* race 1. During the experimental period, plants were watered daily and fertilized with 20-20-20 fertilizer every 2 wk. The green house temperature ranged from 15 to 25°C during the 12 wk that the trial lasted.

Tomato plants were used to propagate the nematodes and then the Hussey and Barker (1973) method was used to extract eggs and juveniles. In this method, roots are cleaned, split in small pieces and washed in a 0.525% sodium hypochlorite (NaClO) solution for 2 min. The roots are then stirred strongly and passed through a 200-mesh sieve (openings 0.149–0.074 mm). The

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Table 1. *Arachis pintoi* accessions charaterized in this study.

PI/CIAT number	Lat. (South)	Long. (West)	Altitude (meter)
476132	16° 08′	47° 12′	690
497541	18° 38′	44° 04′	640
497574	13° 23′	44° 05′	450
604798	16° 18′	46° 58′	630
604799	16° 19′	46° 51′	580
604800	16° 41′	46° 29′	540
604801	16° 42′	46° 25′	560
604803	14° 25′	44° 22′	510
604804	14° 20′	44° 25′	560
604805	16° 59′	45° 57′	570
604807	13° 18′	46° 48′	510
604808	13° 18′	46° 42′	500
604809	13° 02′	46° 45′	610
604810	13° 06′	46° 45′	600
604811	13° 51′	46° 52′	490
604812	14° 28′	46° 29′	500
604813	14° 27′	47° 00′	480
604814	15° 52′	39° 08′	50
604815	15° 49′	47° 58′	1080
604817	18° 38′	44° 04′	630
604856	16° 53′	42° 07′	360
604857	13° 23′	44° 05′	450
604858	15° 26′	47° 21′	700
604859	17° 03′	42° 21′	360
18745	16° 05′	42° 05′	280
20826	-	-	-
22150	22° 55′	47° 05′	510
22152	16° 52′	46° 35′	550
22159	15° 17′	47° 23′	650
22234	13° 14′	46° 44′	463
22256	16° 10′	46° 01′	580
22260	14° 04′	47° 18′	720
22265	-	-	-
22271	16° 04′	44° 01′	1040

eggs and juveniles are collected on a 500-mesh (openings 0.028 mm) sieve placed under the 200-mesh one. Eggs are subsequently rinsed with H_2O, pored to a beaker and water is added to bring the volume to 1000 ml. A sample is taken, placed on a slide, and the number of eggs per ml is estimated by counting under the microscope. Prior to injecting the egg suspension into the soils, the solution was diluted to 300 eggs per ml. This procedure was followed for each one of the three nematodes used in this experiment.

To each container 5 ml of egg suspension was applied, which brings the total eggs per container to 1500 or 10 eggs cm^3 of soil. Application was delivered with a veterinarian surgical syringe, and during the whole process the eggs were kept in continual suspension by a magnetic stirrer.

The experimental design was a randomized complete block, with four replications for *M. arenaria*, and three replications for *M. javanica* and *M. incognita*. A single plant constituted each replication. *Arachis hypogaea* cv. 'Florunner' was used as a susceptible control to verify inoculum viability.

Twelve weeks after inoculation plants were removed from the containers and soil was carefully washed from the roots with tap water. Plants were then placed in a bucket with roots immersed in a 0.25% Phloxine B solution to stain the egg masses. Roots were rated for gall index (GI), gall size (GS), and percent galled area (GA) in a 1–9 scale and after that a damage index (DI) was calculated based on the same parameters (Sharma *et al.*, 1999). DI was calculated by the following equation: DI = (GI+GS+GA)/3. GI, GS, GA and DI scales are presented in table 2.

Number of egg masses (EI) was rated with a 1–9 scale similar to gall index, where 1 represented no egg masses and 9 more than 100 egg masses.

Table 2. Gall Index, gall size, percent galled area and damage index values.

Scale value	Gall index (GI)	Gall size (GS)	Percent galled area (GA)	Damage Index (DI)
1	No galls	No galls	No galls	Highly resistant*
2	1–5 galls			Resistant
3	6–10 galls	10% increase	1–10% root galled	Resistant
4	11–20 galls			Moderate resistant
5	21–30 galls	30% increase	11–30% root galled	Moderate resistant
6	31–50 galls			Susceptible
7	51–70 galls	31–50% increase	31–50% root galled	Susceptible
8	71–100 galls			Highly susceptible
9	>100 galls	>50% increase	>50% root galled	Highly susceptible

*Accessions with DI and/or EI =1, were considered highly resistant; DI and/or EI > 1 and ≤ 3, were classified as resistant; DI and/or EI > 3 and ≤ 5, were classified as moderately resistant; DI and/or EI > 5 and ≤ 7, were classified as susceptible; and finally accessions with DI and/or EI > 7 and ≤ 9, were classified as highly susceptible. If there was discrepancy between DI and EI values, the higher value was applied.

Table 3. Analysis of variance of *Arachis pintoi* germplasm reaction to *M. Arenaria*, *M. javanica* and *M. incognita*.

Meloidogyne arenaria					
		DI†		EM††	
Source	df	MS	Pr>F	MS	Pr>F
Accession	44	8.61	0.0001	8.44	0.0001
Replication	3	0.99	0.5515	0.44	0.7428
Error	78	1.09		1.05	

Meloidogyne javanica					
		DI†		EM††	
Source	df	MS	Pr>F	MS	Pr>F
Accession	39	0.203	0.0054	0.011	0.0002
Replication	2	0.241	0.0905	0.033	0.3986
Error	55	0.096		0.012	

Meloidogyne incognita					
		DI†		EM††	
Source	df	MS	Pr>F	MS*	Pr>F
Accession	39	0.0955	0.0015	-	-
Replication	2	0.0364	0.3969	-	-
Error	48	0.0386		-	

†Damage index
††Egg mass
*No differences were observed

Accessions with EI = 1 were considered highly resistant to nematode reproduction and with EI = 9 were highly susceptible. Intermediate values followed the DI scale.

Results and Discussion

Reaction to *Meloidogyne arenaria*, *M. javanica*, and *M. incognita* was established in accordance with the methodology proposed by Sharma *et al.* (1999). The analysis of variance of *A. pintoi* reaction to *M. Arenaria*, *M. javanica*, and *M. incognita* showed significant ($P < 0.01$) differences among the accessions (Table 3).

M. arenaria reaction of *A. pintoi* germplasm is presented in Table 4. Large genetic variability was observed among the accessions with respect to this characteristic. Among the 44 accessions evaluated, 12 were classified as highly resistant, 14 were classified as resistant, 15 were considered moderately resistant, 2 were considered susceptible, and one was considered highly susceptible. Overall 93% of the accessions presented some level of resistance and only 7% were classified as susceptible.

The *A. pintoi* accessions also demonstrated significant variation ($P < 0.01$) in response to infestation with *M. javanica* (Table 3). Although, significant variation was presented in *M. javanica* reaction, all 39 accessions evaluated were classified as highly resistant or resistant (Table 4).

In the case of *M. incognita* reaction significant differences were observed among accessions only for DI (Table 3). All except two accessions showed no galling or egg mass production (Table 5). Other

Table 4. Reaction of *Arachis pintoi* germplasm to *M. arenaria* race 1 and *M. javanica*.

	M. arenaria (race 1)			*M. javanica*		
Accession (PI/CIAT)	Egg Mass (EM)	Damage Index (DI)	Reaction Grade	Egg Mass (EM)	Damage Index (DI)	Reaction Grade
20826	1.0	1.0	HR*	1.0	1.0	HR
22150	2.3	4.6	MR	1.0	1.0	HR
22151	1.0	1.6	R	1.0	1.0	HR
22152	1.0	1.6	R	1.0	1.0	HR
22154	6.0	5.2	S	1.0	1.0	HR
22159	2.0	4.2	MR	1.0	1.0	HR
22174	2.0	2.5	MR	1.0	1.0	HR
22175	1.0	1.0	HR	1.0	1.0	HR
22232	1.0	1.0	HR	1.0	1.0	HR
22233	1.0	1.0	HR	1.0	1.0	HR
22234	1.0	2.7	R	1.0	1.0	HR
22236	2.0	2.3	MR	1.0	1.0	HR
22238	1.0	1.0	R	1.0	1.0	HR
22241	1.0	1.0	HR	1.0	1.0	HR
22256	2.3	2.7	MR	1.0	1.0	HR
22259	1.0	1.0	HR	1.0	1.0	HR
22265	4.0	7.2	S	1.0	1.0	HR
22268	1.0	1.0	HR	1.0	1.0	HR
22271	3.3	4.8	MR	1.0	1.0	HR
22289	1.0	2.3	R	1.0	1.0	HR
22324	1.0	2.7	R	1.0	1.0	HR
22325	1.8	1.8	MR	1.0	1.0	HR
22339	1.0	2.1	R	1.7	2.1	R
476132	1.0	1.6	R	1.0	1.0	HR
497574	1.0	1.8	R	1.0	1.0	HR
604798	1.0	2.1	R	1.0	1.0	HR
604799	1.0	1.0	HR	1.0	1.0	HR
604800	2.0	3.7	MR	1.0	1.0	HR
604803	2.0	3.7	MR	1.0	1.0	HR
604805	1.3	2.1	MR	1.0	0.7	HR
604808	9.0	7.0	HS	1.0	1.6	R
604809	1.5	1.0	MR	1.0	1.0	HR
604810	1.0	2.7	R	1.0	1.0	HR
604812	2.3	4.3	MR	1.0	1.0	HR
604813	1.0	1.0	HR	1.0	1.0	HR
604815	1.0	1.0	HR	1.0	1.0	HR
604818	1.0	1.0	HR	1.0	1.0	HR
604858	1.0	1.0	HR	1.0	1.0	HR
604859	1.0	3.8	MR	1.0	1.0	HR
Florunner	8.7	7.3	HS	1.0	2.1	R

*HR = Highly resistant; R = Resistant; MR = Moderate resistant; S = Susceptible; HS = Highly susceptible

Table 5. Reaction of *Arachis pintoi* germplasm to *M. incognita* race 1.

Accession (PI/CIAT)	*M. incognita*		
	Egg Mass (EM)	Damage Index (DI)	Reaction Grade
20826	1.0	1.0	HR
22150	1.0	1.0	HR
22151	1.0	1.0	HR
22152	1.0	1.0	HR
22154	1.0	1.0	HR
22159	1.0	1.0	HR
22174	1.0	1.0	HR
22175	1.0	1.0	HR
22232	1.0	1.6	HR
22233	1.0	1.0	HR
22234	1.0	1.0	HR
22236	1.0	1.0	HR
22238	1.0	1.0	HR
22241	1.0	1.0	HR
22256	1.0	1.0	HR
22259	1.0	1.0	HR
22265	1.0	1.0	HR
22268	1.0	1.0	HR
22271	1.0	2.7	R
22289	1.0	1.0	HR
22324	1.0	1.0	HR
22325	1.0	1.0	HR
22339	1.0	1.0	HR
476132	1.0	1.0	HR
497574	1.0	1.0	HR
604798	1.0	1.0	HR
604799	1.0	1.0	HR
604800	1.0	1.0	HR
604803	1.0	1.0	HR
604805	1.0	1.0	HR
604808	1.0	1.0	HR
604809	1.0	1.0	HR
604810	1.0	1.0	HR
604812	1.0	1.0	HR
604813	1.0	1.0	HR
604815	1.0	1.0	HR
604818	1.0	1.0	HR
604858	1.0	1.0	HR
604859	1.0	1.0	HR
Florunner	1.0	1.0	HR

*HR = Highly resistant; R = Resistant; MR = Moderate resistant; S = Susceptible; HS = Highly susceptible

reports have shown that in general *Arachis pintoi* have near immunity to *M. incognita*. In fact, *A. hypogaea* is used as a non-host differential for *M. incognita* in the standard test to characterize populations of root-knot nematodes into major species and races. *A. hypogaea* is however generally susceptible to *M. arenaria*.

Nematode resistance is a valuable attribute for any species that will be incorporated into agriculture systems. It is more important with perennial plants that will have long-term exposure to soil borne problems. For a forage crop, nematode susceptibility can affect the ability to persist over a long period in the pasture. In the case of *A. pintoi*, which is known as multiple use legume, this characteristic could improve its utilization as ground cover and in crop rotations with cultures that are susceptible to root-knot nematodes. This is the case of the common peanut planted in the southeastern USA, which requires a crop rotation with bahiagrass (Paspalum notatum). The introduction of *A. pintoi* in bahiagrass pasture could improve nematode control and additionally improve the nutritive value of the pasture.

In the case of *A. pintoi*, nematode resistance is remarkably important to permit a wide use of the species as forage crop or even as a cover crop. Also this is important due to the fact that the species could be considered a useful source of genes for *A. hypogaea*, which is worldwide cultivated. Since direct crossing among the two species is not possible, some authors include *A. pintoi* in the tertiary gene pool of *A. hypogaea*. However, with the recent progress of molecular biology tools, direct transfer could be achieved even for non-related species of the genus, which makes this source of resistance potentially important.

Even though, knowledge about sources of nematode resistance is extremely important to the general use of the species and for its use in breeding programs of *A. hypogaea*, little was know about *A. pintoi* germplasm accessions response to root-knot nematodes. Information available is usually restricted to one or a few accessions. Sharma *et al.* (1999) studied *M. javanica* race 3 reaction of 161 accessions of wild *Arachis* species.

They reported that of the nine accessions of *A. pintoi* evaluated, eight were considered susceptible or highly susceptible, but a single accession was classified as moderately resistant. By contrast, all *A. pintoi* accessions were highly resistant to the M. javanica population used in this research (Not classified as a race).

Queneherve et al. (2002) examined *A. pintoi* reaction to *Radopholus similis*, *Pratylenchus coffeae*, *Hoplolaimus seinhorsti*, *Meloidogyne incognita* and *M. mayaguensis*. Forty-five days after inoculation *R. similis*, *H. seinhorsti* and *P. coffeae* multiplied in the roots. *A. pintoi* did not allow the multiplication of *M. incognita* and *M. mayaguensis*, indicating the inability of *A. pintoi* to act as a host to these two root-knot nematodes.

Santiago *et al.* (2002) investigated the *A. pintoi* reaction to *M. paranaensis* and *M. incognita* races 1, 2, 3, and 4. They reported that no root

penetration by *M. incognita* and *M. paranaensis* juveniles had occurred, and hence there was no gall or egg mass formation. They concluded that in general *A. pintoi* accessions had an antagonistic effect on the nematodes, suggesting that they could be used as an interccrop or cover crop to reduce *M. paranaensis* and *M. incognita* populations. This research supports this conclusion and includes populations of *M. arenaria* since many accessions presented resistance to this species.

Nelson *et al.* (1989) evaluated the resistance to *M. arenaria* of 116 wild *Arachis* spp. genotypes, including a single *A. pintoi* accession. Resistance was identified in accessions from 11 of 15 wild species tested and in 10 of 20 accessions belonging to undescribed species. Results of field and greenhouse experiments were similar; 26 of 31 accessions common to both tests gave similar responses in both tests. Among these species, the authors identified *A. batizocoi* Krapov. & W.C. Gregory and *A. cardenasii* Krapov. & W.C. Gregory as species that are both resistant to *M. arenaria* and compatible with *A. hypogaea*. These sources of *M. arenaria* resistance were used to develop the germplasm line TxAG-6 (Simpson *et al.*, 1993).

TxAG-6 is a amphiploid formed by first crossing *A. cardenasii*/*A. diogoi* Hoehne, and then crossing the 50% pollen fertile F1 hybrid with *A. batizocoi*. The resulting tri-species hybrid ($2n = 20$) was $<1\%$ pollen stained and produced no fruit. The chromosome number was doubled with colchicine to form TxAG-6 (Simpson *et al.*, 1993).

TxAG-6 is about 89% pollen stained and is highly fertile, both selfed or when crossed with *A. hypogaea*. The fertile amphiploid was crossed with Florunner to incorporate M. arenaria resitance, and five back-crosses later produced the designated breeding line, TP262-3-5, which was later denominated cultivar 'Coan' (Simpson and Starr, 2001). In each backcross cycle, selection was made for agronomic characters similar to Florunner and resistance to root-knot nematodes (Nelson *et al.*, 1990; Starr *et al.*, 1990).

TxAG-6 was also used to produce the breeding line TP301-1-8, which resulted from seven back-crosses with Florunner. In each generation, selection was made for agronomic characters matching those of Florunner, the recurrent parent, and for resistance to root-knot nematodes. TP301-1-8 was named and register as cultivar 'NemaTAM' (Simpson *et al.*, 2003).

Information published and available seems to support the results obtained in this research with respect to nematode reaction of *A. pintoi*. The source of resistance of these accessions could be used in breeding programs of *A. hypogaea* and more important qualify *A. pintoi* as potential forage, at least by this criteria, in environments where nematode infestation is a factor. Another positive outcome of this result is the ability of the species to suppress the multiplication of nematodes, and then be an important cover crop to species with nematode susceptibility problems.

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