RESISTANCE TO *MELOIDOGYNE JAVANICA* RACE 3 IN THE *ARACHIS* GENE POOL

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Summary. Forty six accessions of wild *Arachis* spp. in sections *Arachis, Procumbentes, Heteranthae, Erectoides*, and *Caulorhizae* were evaluated in a glasshouse for resistance to *Meloidogyne javanica* race 3. Based on numbers and size of galls, galled area of root, and numbers of egg masses, two accessions of *A. cardenasii* (ICG 13237, ICG 15175) in section *Arachis* and three accessions of *A. sylvestris* (ICG 15142, ICG 15147, ICG 15164) in section *Heteranthae* were highly resistant. The origin of these accessions was in Bolivia (ICG 15175, ICG 13237) and Brazil (ICG 15142, ICG 15147, ICG 15164). Gall and egg mass ratings of all the accessions indicated that resistance varied within species. Of the eight *A. duranensis* accessions tested, one was resistant (ICG 13183), one moderately resistant (ICG 8203), three susceptible (ICG 13185, ICG 13194, and ICG 13201) and three highly susceptible (ICG 13206, ICG 13217, and ICG 13218). There were large differences in gall number, size, and egg mass number on some accessions; accession ICG 15141 (*A. villosa*) had 15-20 galls and 1-5 egg masses while ICG 15172 (*A. glandulifera*) had more galls than egg masses. All the tested accessions of sections *Procumbentes, Caulorhizae* and *Erectoides* were susceptible.

Peanut (*Arachis hypogaea* L.) or groundnut is an important annual legume crop grown in about 108 countries with a total estimated area of 23.2 million ha and production in shell of 31.0 million t during the 1998 crop season (FAO, 1999). The crop is grown at both subsistence and commercial levels of farming mainly as a source of edible oil and protein. Plant-parasitic nematodes reduce the groundnut crop yields by damaging pegs, pods, and seeds and by feeding on roots and weakening the plants. Although more than 100 nematode species have been reported to be associated with groundnut, only a few species are reported to cause economic losses in pod yield, especially in the tropics (Sharma and McDonald, 1990). Worldwide, annual yield losses due to nematodes in groundnut are estimated to be US $ 1 billion (ICRISAT 1992; Sasser and Freckman, 1987). The losses due to nematodes are much higher in the developing tropical countries where some of the highly damaging populations are widespread and farmers are not aware of the nematode induced crop damage. In several countries in West Africa, plant parasitic nematodes in conjunction with other soil disorders have constrained the farmers from raising acceptable peanut crops (Germani, 1981; Sharma *et al.*, 1992). The root-knot nematodes (*Meloidogyne* spp.) are a potential threat to sustainable production of peanut in some of the cropping areas of India and China (Song *et al.,* 1996; Sree Latha *et al.,* 1998; Di Vito *et al.,* 1999).

One of the objectives of nematology research activities in the Genetic Resources and Enhancement Program at ICRISAT, Patancheru is to identify sources of resistance to the important root-knot and cyst nematode species both in the sexually cross-compatible and incompatible wild relatives of pigeonpea [*Cajanus cajan* (L.) Millspagh], chickpea (*Cicer arietinum* L.) and peanut (*Arachis hypogaea*). Previous attempts to discover sources of root-knot nematode resistance in the cultivated germplasm of these legumes were unsuccessful. More than 2500 accessions of cultivated peanut germplasm were screened for resistance to *M. javanica* race 3 but sources with good levels of resistance were not found. However, wild relatives of peanut (Sharma *et al.,*1999) and pigeonpea (Sharma,1995) were found to have resistance genes to important nematode species. The *Arachis* species are reservoirs of useful genes and their genetic potential in crop improvement is known (Nelson *et al.,* 1989; Nelson *et al.,* 1990; Sharma *et al.,* 1999). Accessions with genes for nematode resistance have been located in the genepool of wild *Arachis* species. Some species such as A. *cardenasii* and *A. stenosperma* are cross compatible with *A. hypogaea* and genes conferring nematode resistance to three *Meloidogyne* species (*M. arenaria, M. javanica* and *M. hapla*) are available in the genepool of wild species (Nelson *et al.,* 1989; Sharma *et al.,* 1999).

This paper presents the results of evaluation of accessions of *Arachis* species to identify promising sources of resistance to *M. javanica* race 3, which has emerged as one of the most important nematode parasites of peanut in India with severe crop losses expected in infested areas (Sakhuja and Sethi, 1985; Patel *et al.*, 1988; Sharma and Ashokkumar, 1991; Ali, 1997; Di Vito *et al.,* 1999).

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MATERIAL AND METHODS

Seeds of 46 accessions within 19 *Arachis* species and five botanical sections (*Arachis, Erectoides, Heteranthae,* and *Procumbentes*, and *Caulorrhizae*) were obtained from the Genetic Resources Division of ICRISAT. The seed germination was good in 38 accessions whereas in other accessions it was unsatisfactory. The details of 38 wild species accessions, belonging to four sections, that were successfully evaluated for nematode resistance are given below:

Section *Arachis*: 27 accessions [indicated in parentheses] of 10 species i.e. Ara*chis batizocoi* Krapov. *et* W.C. Gregory [1], *A. benensis* Krapov., W.C. Gregory *et* C.E. Simpson [1], *A. cardenasii* Kraapov. *et* Rigoni [2], *A. correntina* (Burkart) Krapov. *et* W.C. Gregory [1], *A. decora* Krapov., W.C. Gregory *et* Valls [2], *A. duranensis* Krapov. *et* W.C. Gregory [8*], A. glandulifera* Stalker [1], *A. villosa* Benth [2], *A. kuhlmannii* Krapov. *et* W.C. Gregory [6], and *A. stenosperma* Krapov. *et* W.C. Gregory [3]; Section *Erectoides*: 3 accessions of *A. hermannii* Krapov. *et* W.C. Gregory [1], *A. paraguariensis* Chodat *et* Hassl [1] and *A. major* Krapov. *et* W.C. Gregory [1]; Section *Heteranthae:* 6 accesions of *A. dardani* Krapov. *et* W.C. Gregory [1], *A. sylvestris* (A. chev.) A. chev. [4] and *A. pusilla* Benth.[1]; Section *Procumbentes:* 2 accessions of *A. appressipila* Krapov. *et* W.C. Gregory [1], and *A. subcoriacea* Krapov. *et* W.C. Gregory [1].

A population of *M. javanica* (Treub) Chitw. race 3, originally collected from the Pallipalem area in Prakasam district of Andhra Pradesh in southern India was cultured on peanut (*Arachis hypogaea*) cultivars JL 24 and TMV 2 in 30-cm-diam. pots in a glasshouse at ICRISAT, Patancheru, Andhra Pradesh, India. The race of *M. javanica* was determined on the basis of differential host reaction (Sharma *et al.*, 1995)

Eggs of *M. javanica* race 3 were extracted from heavily galled roots of 8-week-old peanut cultivars by treatment with sodium hypochlorite (Hussey and Barker, 1973). Five thousand nematode eggs in water suspension were placed in the same depressions in which seeds were sown. All the accessions were evaluated in 5 cm pots filled with 2 parts of red soil:1 part of sand and kept in a glasshouse (maximum temperature between 22 and 33 ^{0}C and minimum temperature between 20 and 23 ^{0}C). Pots, arranged in completely randomized design, were irrigated daily. Number of plants studied for each accession varied from 10 to 18. A nematode susceptible cultivar ICG 799 (cv. Kadiri 3) was included as control. Eight weeks after seedling emergence, roots were carefully washed with tap water and evaluated for number of egg masses and galls, gall size, and percent galled area of root. Nematode reproduction was measured by counting egg masses. Roots were treated with 0.25% trypan blue to stain the egg masses (Sharma and Mohiuddin, 1993). Roots were rated on a 1-9 scale for gall index (GI): 1 = no galls; 2 = 1-5 galls; 3 = 6-10 galls; 4 = 11-20 galls; 5 = 21-30 galls; 6 = 31-50 galls; 7 = 51-70 galls; 8 = 71-100 galls; and 9 = >100 galls. Gall size (GS) was evaluated on a 1-9 scale (1 = no galls; 3 = very small, about 10% increase in root area at the galled region over non-galled normal root area; 5 = small galls, about 30% increase; 7 = medium, about 31-50 % increase; 7 = 31-50% root area galled; and 9 = big galls, about 51-100% increase). Percent galled area (GA) of root was rated on a 1-9 scale (where 1 = no galls; 3 = 1-10% root area galled; 5 = 11-30% root area galled; 7 = 31-50% root area galled; and 9 = > 50% root area galled). G1, GS and GA are intrinsic components of damage by the root-knot nematodes and these were given equal importance in assessing the nematode induced damage and a damage index (DI) was calculated by dividing the sum of GI, GS and GA by three (GI + GS + GA/3). Accessions with DI = 1 were considered highly tolerant to damage, with DI = 2-3 as resistant, with DI = 4-5 as moderately tolerant, with DI = 6-7 as susceptible, and with DI = 8-9 as highly susceptible to damage. Numbers of egg masses were rated using the 1-9 scale for gall number (Egg mass index (EI) 1 = no egg masses, 9 = >100 egg masses). Accessions with EI = 1 were considered highly resistant to nematode reproduction and with EI = 9 were considered highly susceptible. Average egg sac and damage indices and standard errors for each accession were calculated.

RESULTS AND DISCUSSION

Five accessions were highly resistant to nematode reproduction as well as tolerant to damage (Table I); nine accessions were resistant, and another nine moderately resistant. There was no plant-to-plant variation in the egg sac and damage indices of all the five highly resistant accessions. The standard error of means for EI ranged from 0.0 to 0.3 and for DI it ranged from 0.0 and 0.2 for resistant and moderately resistant accessions. Of the remaining accessions ten were susceptible and five wild species and the control cultivar were highly susceptible. In section *Arachis*, two accessions of *A. cardenasii* (ICG 13237 and ICG 15175) were highly resistant. An accession each of *A. duranensis* (ICG 13183), *A. villosa* (ICG 13258), *A. glandulifera* (ICG 15172) and two accessions of *A. stenosperma* (ICG 15157 and ICG 15237) were resistant. The galled area of these accessions was very low (GA = 1-2). Some differences were evident even within these resistant accessions; the number of galls was greater on ICG 15157 (GI = 3.0) compared with that on ICG 15172 (GI = 1.1). The size of the gall was smallest on ICG 15172. Egg masses were generally not found on ICG 13258 (*A. villosa*) and ICG 15237 or 15160 (*A. stenosperma).* A wide range of reactions of different accessions even within a species was observed. For example, out of eight accessions of *A. duranensis* tested, one accession (ICG 13183) was resistant, another accession (ICG 8203)

Table I. Reaction of *Arachis* species accessions to *Meloidogyne javanica* Race 3.

ICG (a)	Synonym(b)	Section (c)	Species	Origin	Egg sac Index (d) Mean (± S.E)	Damage Index (e) Mean (± S.E)
Accessions with DI or EI = 1 (Highly resistant)						
13237	PI 475994	*Arachis*	*A. cardenasii*	Bolivia	1.0 ± 0.0	1.0 ± 0.0
15142	PI 476136	*Heteranthae*	*A. sylvestris*	Brazil	1.0 ± 0.0	1.0 ± 0.0
15147	PI 497543	*Heteranthae*	*A. sylvestris*	Brazil	1.0 ± 0.0	1.0 ± 0.0
15164	–	*Heteranthae*	*A. sylvestris*	Brazil	1.0 ± 0.0	1.0 ± 0.0
15175	PI 476001	*Arachis*	*A. cardenasii*	Bolivia	1.0 ± 0.0	1.0 ± 0.0
Accessions with DI or EI < 3 and > 1(Resistant)						
13160	PI 468328	*Arachis*	*A. batizocoi*	Bolivia	1.9 ± 0.0	2.7 ± 0.2
13183	PI 497267	*Arachis*	*A. duranensis*	Argentina	2.0 ± 0.2	2.2 ± 0.3
13258	–	*Arachis*	*A. villosa*	–	1.0 ± 0.1	1.3 ± 0.1
15141	–	*Arachis*	*A. villosa*	Uruguay	1.6 ± 0.1	2.8 ± 0.1
15149	–	*Erectoides*	*A. paraguariensis*	Brazil	2.6 ± 0.2	2.2 ± 0.2
15157	–	*Arachis*	*A. stenosperma*	Brazil	2.0 ± 0.1	2.3 ± 0.1
15160	–	*Arachis*	*A. stenosperma*	Brazil	1.0 ± 0.0	2.6 ± 0.0
15172	PI 468336	*Arachis*	*A. glandulifera*	Bolivia	1.2 ± 0.1	1.1 ± 0.2
15237	–	*Arachis*	*A. stenosperma*	Brazil	1.0 ± 0.2	1.9 ± 0.0
Accessions with DI or EI > 3 and < 5 (Moderately resistant)						
8203	PI 475847	*Arachis*	*A. duranensis*	Bolivia	4.0 ± 0.2	3.5 ± 0.1
13227	–	*Heteranthae*	*A. dardani*	Brazil	3.2 ± 0.1	2.8 ± 0.1
13251	–	*Erectoides*	*A. hermannii*	Brazil	3.0 ± 0.0	3.3 ± 0.3
13262	–	*Erectoides*	*A. major*	Brazil	3.5 ± 0.2	4.5 ± 0.2
14864	–	*Arachis*	*A. kuhlmannii*	Brazil	4.2 ± 0.2	5.3 ± 0.3
14866	–	*Arachis*	*A. kuhlmannii*	Brazil	5.0 ± 0.3	5.0 ± 0.2
14919	PI 476109	*Arachis*	*A.kuhlmannii*	Brazil	3.3 ± 0.2	3.5 ± 0.1
14920	PI 497542	*Heteranthae*	*A. sylvestris*	Brazil	5.0 ± 0.1	4.3 ± 0.1
15145	PI 476126	*Arachis*	*A. kuhlmannii*	Brazil	4.0 ± 0.2	4.1 ± 0.2
8918	–	*Arachis*	*A. correntina*	Argentina	3.7 ± 0.3	5.1 ± 0.1
13185	PI 497269	*Arachis*	*A. duranensis*	Argentina	5.6 ± 0.0	6.5 ± 0.0
13194	PI 497266	*Arachis*	*A. duranensis*	Argentina	6.8 ± 0.4	6.7 ± 0.2
13201	PI 497267	*Arachis*	*A. duranensis*	Argentina	6.0 ± 0.0	6.5 ± 0.1
14880	PI 591349	*Heteranthae*	*A. pusilla*	Brazil	6.8 ± 0.1	5.2 ± 0.1
14939	–	*Arachis*	*A. decora*	Brazil	6.3 ± 0.0	4.5 ± 0.2
15144	–	*Arachis*	*A. kuhlmannii*	Brazil	6.4 ± 0.2	5.4 ± 0.0
15146	PI 476126	*Arachis*	*A. kuhlmannii*	Brazil	5.6 ± 0.5	4.7 ± 0.2
15156	–	*Procumbentes*	*A. subcoriacea*	Brazil	6.6 ± 0.0	6.0 ± 0.0
15158	–	*Procumbentes*	*A. appressipila*	Brazil	6.6 ± 0.1	4.6 ± 0.0
13206	PI 497269	*Arachis*	*A. duranensis*	Argentina	6.6 ± 0.1	7.5 ± 0.2
13215	PI 475879	*Arachis*	*A. benensis*	Bolivia	9.0 ± 0.0	9.0 ± 0.1
13217	PI 475887	*Arachis*	*A. duranensis*	Argentina	7.4 ± 0.5	6.9 ± 0.2
13218	PI 475886	*Arachis*	*A. duranensis*	Argentina	8.4 ± 0.7	7.7 ± 0.2
14946	–	*Arachis*	*A. decora*	Brazil	9.0 ± 0.1	7.0 ± 0.0
799 (Control)	–	*Arachis*	*A. hypogaea*	India	9.0 ± 0.0	9.0 ± 0.0

(a) ICRISAT (International Crops Research Institute for the Semi Arid Tropics) germplasm accession number.
(b) Refers to the identity of these accessions in the USDA peanut germplasm bank.
(c) Members of section *Arachis* are cross compatible with the cultivated species, *A. hypogaea* and others are incompatible.
(d) EI (Egg sac index): 1 = no egg masses, 2 = 1 to 5, 3 = 6 to 10, 4 = 11 to 20, 5= 21 to 30, 6 = 31 to 50, 7 = 51 to 70, 8 = 71 to 100, 9 = >100.
(e) DI (Damage Index): (gall index + gall size + galled area) /3.

moderately resistant, three accessions (ICG 13185, ICG 13194, and ICG 13201) susceptible, and three other accessions (ICG 13206, ICG 13217, and ICG 13218) highly susceptible. Three accessions, ICG 11563 *(A. cardenasii)*, ICG 13183 (*A. duranensis*), and ICG 13188 (*A. stenosperma*) showed resistance reactions but this could not be confirmed because of poor germination of the seed (data not shown). Some distinct differences were observed in the reactions of different accessions. For example, ICG 13160 (*A. batizocoi*) showed greater root damage in terms of galling on roots despite limited reproduction of the nematode. In section *Erectoides,* the two accessions, ICG 15149 (*A. paraguariensis*) and ICG 13262 (*A. major*) were resistant and moderately resistant, respectively. The root area covered with galls on ICG 15149 was very small. Plants of ICG 13262 showed variation in the number of galls and egg masses per plant. In section *Caulorrhizae*, only one accession (ICG 14888) of *A. pintoi* was tested. The seed germination was poor, but the plants were highly susceptible to nematode damage and reproduction (data not shown). In section *Heteranthae*, three accessions of *A. sylvestris* (ICG 15142, ICG 15147, and ICG 15164) were highly resistant. Another accession also showed a highly resistant response but seed germination was poor and the results could not be confirmed in repeat tests. ICG 14920 was susceptible and 51-70 small galls were observed on the plants. Two accessions (ICGs 13227 and 15166) of *A. dardani* and one accession (ICG 14880) of *A. pusilla* were susceptible. In Section *Procumbentes*, accessions of *A. appressipila* (ICG 15158) and *A. subcoriacea* (ICG 15156) were susceptible. The number of galls was much greater on ICG 15156 than on ICG 15158. The size of the galls on ICG 15158 was smaller than that on ICG 15156.

The results confirm the presence of high levels of resistance to root-knot nematodes in the wild relatives of peanut. In a previous study (Sharma *et al.*, 1999), six out of seven highly resistant accessions were from Brazil and in the present study also three out of five accessions were from Brazil. Though many accessions from Brazil were susceptible, it may still be useful to evaluate the *A. hypogaea* germplasm collected from Brazil for the likely presence of resistance to the nematode. *A. sylvestris* (ICG 15147) and *A. villosa* (ICG 13258) which were found to be highly resistant and resistant, respectively to race 3 in the present study, were previously reported as resistant to *M. arenaria* race 1 (Nelson *et al.*, 1989). Similarly, *A. batizocoi* (ICG 13160) has been reported to be resistant to web blotch (*Didymella arachidicola*) (Subrahmanyam *et al.*, 1985). The identified nematode resistant accessions should be tested for resistance to other root-knot nematode species and other diseases and pests to identify multiple resistances. *A. cardenasii* (ICG 13237), which is found to be highly resistant and *A. batizocoi* (ICG 13160), *A. duranensis* (ICG 13183), *A. villosa* (ICG 13258 and 15141), and *A. stenosperma* (ICGs 15157, 15160, and 15237) which were resistant in the present study are cross compatible with the cultivated species, *A. hypogaea* and they can be utilized in conventional breeding programmes aimed at developing nematode resistant cultivars. Further investigations on mechanisms of resistance in these accessions will be useful to understand the nature of resistance.

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