Resistance to *Rotylenchulus reniformis*, *Heterodera cajani*, and *Meloidogyne javanica* in Accessions of *Cajanus platycarpus*

S. B. Sharma, Scientist (Nematology), International Crops Research Institute for the Semi-Arid Tropics, ICRISAT Asia Center, Patancheru, Andhra Pradesh 502 324, India

ABSTRACT

Sharma, S. B. 1995. Resistance to *Rotylenchulus reniformis, Heterodera cajani,* and *Meloidogyne javanica* in accessions of *Cajanus platycarpus*. Plant Dis. 79:1033-1035.

Rotylenchulus reniformis, Heterodera cajani, and *Meloidogyne javanica* are important nematode pathogens of pigeonpea (*Cajanus cajan*), a legume crop cultivated widely in the semiarid tropics. Accessions of *Cajanus platycarpus*, a wild relative of pigeonpea, were evaluated in the greenhouse for resistance to *R. reniformis* race A, *H. cajani,* and *M. javanica* races 1 and 3. Two accessions, ICPWs 60 and 62, were resistant to *R. reniformis* race A. ICPWs 62, 69, and 70 were resistant to *H. cajani*. All the accessions were susceptible to *M. javanica* races. Race 1 produced bigger galls than those produced by race 3. ICPW 64 had lower damage indices (7.7 for race 1, and 5.4 for race 3) than other accessions. ICPW 62 was the only accession with resistance to two nematode species, *H. cajani* and *R. reniformis*. Seed of ICPWs 62, 69, and 70 with high levels of resistance to *H. cajani* were collected and multiplied. These lines will be useful in breeding programs as well as in research to obtain an understanding of the mechanism of resistance.

Additional keyword: multiple resistance

Wild relatives of cultivated plant species have contributed useful genes for resistance to pests and diseases (19). Nematode-resistant accessions of wild relatives of potato, tobacco, and tomato have been successfully used as sources of genes for resistance to cyst and root-knot nematodes (2). Pigeonpea (*Cajanus cajan* (L.) Millsp.) is a principal legume crop of subsistence farming systems in many parts of the semiarid tropics and is a host of a large number of plant-parasitic nematode species (6). Although nematodes have been reported associated with pigeonpea in more than 24 countries, only a few species are recognized as important pathogens (17). The reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira, the cyst nematode, *Heterodera cajani* Koshy, and the root-knot nematode, *Meloidogyne javanica* (Treub) Chitwood are among the pathogens of pigeonpea; they adversely affect pigeonpea growth and reduce plant biomass and grain yield in many pigeonpea-growing regions (12,14,17). No pigeonpea cultivars with resistance to any of these nematode species have been developed. Researchers at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Asia Center, Andhra Pradesh, India, are screening the pigeonpea genebank to identify sources of resistance. Accessions of wild relatives of pigeonpea are reservoirs of useful genes (5,7,8,9). Genes for resistance to *H. cajani*, *R. reniformis*, and *M. javanica* are available in accessions of wild relatives of pigeonpea (15,16).

Cajanus platycarpus (Benth) van der Maesen, a wild relative of pigeonpea, has some useful genes not present in the pigeonpea gene pool, with attributes that include early flowering and maturity, photoperiod insensitivity, profuse flowering and pod setting, high harvest index, annuality, and quick seedling growth (3,7,8, 20). Thirteen accessions of this species, collected from a wide eco-geographical range, are in the gene bank at ICRISAT. Some of these accessions are sources of resistance to *Phytophthora* blight (8). Because resistance to important nematode species is an attribute that should be considered when selecting accessions of *C. platycarpus* for hybridization with pigeonpea, the objective of this project was to probe the available accessions for resistance to *R. reniformis, H. cajani,* and *M. javanica.*

Submitted as Journal Article JA No. 1744 by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India.

Accepted for publication 20 April 1995.

MATERIALS AND METHODS

Seeds of 13 accessions of *C. platycarpus* were obtained from the Genetic Resources Division of ICRISAT. Pigeonpea genotype ICPW (ICRISAT germ plasm number) 237 was the susceptible check for *M. javanica*, and ICPL 87 for *R. reniformis* and *H. cajani* (10,11,15,16). The following conditions were common to all tests: all seeds were mechanically scarified to promote germination; evaluations were conducted in a greenhouse (maximum temperature between 25 and 32°C and minimum temperature between 20 and 23°C); pots were arranged in a completely randomized design; and plants were irrigated daily with tap water and weekly with quarter-strength Arnon's nutrient solution (1).

Nematode populations. Isolates of *R. reniformis* race A, which reproduces on castor, cowpea, and cotton, and *H. cajani* were increased on pigeonpea cultivar ICPL 87. *Meloidogyne javanica* races 1 and 3 (18) were increased on tomato (*Lycopersicon esculentum*) cultivar Rutgers.

Screening for resistance to *R. reniformis*. Four seeds of each accession of *C. platycarpus* were sown in each of five 15-cm-diameter pots filled with 1,700 cm^3 autoclaved sandy clay loam soil (60% sand, 7% silt, 33% clay; pH 5.9) infested with 10 to 15 vermiform stages of *R. reniformis* per cm^3 soil. Within 5 to 6 weeks of seedling emergence, plants were gently removed from the pots, and the roots were dipped for 3 min in 0.25% trypan blue and washed with tap water to remove excess stain (10). All the egg masses were colored blue, and roots were not stained. Egg masses per root were counted and an egg mass index (EI) was assigned based on a 1 (highly resistant) to 9 (highly susceptible) scale, as follows: 1 = no egg masses; 2 = 1 to 5 egg masses; 3 = 6 to 10 egg masses; 4 = 11 to 15 egg masses; 5 = 16 to 20 egg masses; 6 = 21 to 30 egg masses; 7 = 31 to 40 egg masses; 8 = 41 to 50 egg masses; and 9 = >50 egg masses.

Screening for resistance to *H. cajani*. An autoclaved sand + vertisol (silty clay loam, 39% sand, 20% silt and 41% clay; pH 8.0) mixture (3:1, vol/vol) infested with 12 eggs and juveniles of *H. cajani* per cm^3 soil was added to 15-cm-diameter plastic pots. For each *C. platycarpus* accession, there were four pots each sown with four seeds. Thirty-five days after seedling emergence, roots were gently washed with tap water and the number of white females of *H. cajani* on the roots were counted (11). Plants were evaluated on a 1 (highly resistant), ≤3 (resistant), ≤5 (moderately resistant), ≤7 (susceptible) and ≤9 (highly susceptible) female index (FI) on the basis of the number of females on each root as follows: 1 = no females; 3 = 1 to 5; 5 = 6 to 10; 7 = 11 to 30; and 9 = >30 females (11). Reactions of all accessions were confirmed in a repeat test.

Screening for resistance to *Meloidogyne javanica*. In separate greenhouse experiments, eggs of races 1 and 3 were extracted with NaOCl (4) from 8-week-old

cultures maintained on tomato. For each *C. platycarpus* accession, there were five 15-cm-diameter pots, filled with autoclaved sandy loam soil, each sown with three seeds. Five thousand nematode eggs in water suspension were placed in the same depressions in which seeds were sown. Eight weeks after seedling emergence, roots were carefully washed with tap water and evaluated as follows: gall index (GI) was rated on 1 to 9 scale with 1 = no galls, 2 = 1 to 5 galls, 3 = 6 to 10, 4 = 11 to 20, 5 = 21 to 30, 6 = 31 to 50, 7 = 51 to 70, 8 = 71 to 100, and 9 = >100. Gall size (GS) was evaluated on a 1 to 9 scale as follows: 1 = no galls; 3 = very small, approximately 10% larger in diameter than nongalled normal root; 5 = small galls, 30% larger; 7 = medium, 31 to 50% larger; and 9 = big galls, 51 to 100% larger. Percent galled area (GA) was rated on a 1 to 9 scale as follows: 1 = no galls; 3 = 1 to 10% root area galled; 5 = 11 to 30%; 7 = 31 to 50%; and 9 = >50% (16). GI, GS, and GA are intrinsic components of damage by the root-knot nematodes and these were given equal weight in assessing the damage caused by the nematode (16). A damage index (DI) was calculated as (GI + GS + GA)/3. Accessions with DI = 1 were considered highly resistant; DI = 2 to 3 were resistant; DI = 4 to 5, moderately resistant; DI = 6 to 7, susceptible; and DI = 8 to 9, highly susceptible. Nematode reproduction was measured as the number of egg masses. Roots were treated with 0.25% trypan blue to stain the egg masses blue (13). An egg mass index (EI) was assigned based on the same 1 to 9 scale used for gall number (1= no egg masses, 9 = >100 egg masses). Accessions with EI = 1 were considered highly resistant to nematode reproduction whereas those with EI = 9 were highly susceptible.

Table 1. Host suitability of *Cajanus platycarpus* accessions for *Heterodera cajani* and *Rotylenchulus reniformis* race A

	R. reniformis			*H. cajani*		
ICPW numbers[a]	No. of plants screened	EI[b]	SE	No. of plants screened	FI[c]	SE
60	61	2.7	0.28	26	7.2	0.26
61	34	5.4	0.44	25	7.2	0.13
62	57	1.3	0.07	24	2.2	0.20
63	57	4.1	0.18	28	8.3	0.18
64	32	4.6	0.24	29	7.5	0.26
65	32	6.6	0.24	26	6.2	0.20
67	32	6.6	0.24	27	7.7	0.28
68	58	5.4	0.37	24	7.7	0.26
69	32	5.5	0.31	28	1.8	0.21
70	32	7.0	0.26	31	2.5	0.26
71	32	6.1	0.25	26	7.7	0.25
72	57	3.4	0.20	28	6.2	0.50
ICPL 87	47	8.5	0.19	24	9.0	0.00

[a] ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) germ plasm accession number.
[b] EI (egg mass index): 1 = no egg masses; 2 = 1 to 5; 3 = 6 to 10; 4 = 11 to 15; 5 = 16 to 20; 6 = 21 to 30; 7 = 31 to 40; 8 = 41 to 50; and 9 = >50 egg masses.
[c] FI (female index): 1 = no female; 3 = 1 to 5; 5 = 6 to 10; 7 = 11 to 30; and 9 = >30 females.

Table 2. Reaction of *Cajanus platycarpus* accessions to *Meloidogyne javanica* races 1 and 3

ICPW nos.[a]	No. of plants screened	Gall index[b]		Gall size[c]		Galled area[d]		Damage index[e]		Egg mass index[f]	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
M. javanica race 1											
60	13	9.0	0.00	6.4	0.27	9.0	0.00	8.1	0.09	9.0	0.00
61	13	8.7	0.21	5.9	0.29	8.8	0.17	7.8	0.17	8.8	0.17
62	13	9.0	0.00	6.2	0.28	8.4	0.27	7.8	0.17	9.0	0.00
63	13	9.0	0.00	6.5	0.24	7.8	0.28	7.8	0.13	9.0	0.00
64	13	8.9	0.00	6.4	0.27	7.9	0.29	7.7	0.14	9.0	0.00
65	13	9.0	0.00	8.5	0.24	9.0	0.00	8.8	0.08	9.0	0.00
66	13	9.0	0.00	8.4	0.27	9.0	0.00	8.8	0.09	9.0	0.00
67	13	9.0	0.00	6.2	0.28	9.0	0.00	8.1	0.09	9.0	0.00
68	13	9.0	0.00	6.4	0.27	9.0	0.00	8.1	0.09	9.0	0.00
69	13	9.0	0.00	7.6	0.27	9.0	0.00	8.5	0.09	9.0	0.00
70	13	9.0	0.00	9.0	0.00	9.0	0.00	9.0	0.09	9.0	0.00
71	13	9.0	0.00	7.8	0.28	9.0	0.00	8.6	0.09	9.0	0.00
72	13	9.0	0.00	6.5	0.24	8.5	0.24	8.0	0.16	9.0	0.00
237	13	9.0	0.00	8.5	0.24	9.0	0.00	8.8	0.11	9.0	0.00
M. javanica race 3											
60	20	7.6	0.24	5.9	0.27	6.8	0.35	6.8	0.21	8.2	0.30
61	22	7.4	0.22	5.3	0.24	7.0	0.33	6.6	0.22	8.8	0.38
62	23	7.6	0.21	6.4	0.36	6.9	0.41	7.0	0.29	8.5	0.16
63	26	8.3	0.18	6.5	0.34	8.1	0.28	7.6	0.19	8.9	0.05
64	21	6.6	0.26	5.0	0.24	4.6	0.32	5.4	0.23	7.3	0.31
65	20	8.2	0.25	5.5	0.21	8.1	0.36	7.3	0.19	8.9	0.18
66	20	8.2	0.22	5.8	0.22	5.8	0.27	6.6	0.16	8.2	0.32
67	15	7.6	0.22	5.7	0.22	6.0	0.27	6.4	0.16	9.0	0.00
68	23	7.5	0.25	5.6	0.31	6.0	0.37	6.5	0.27	9.0	0.00
69	26	8.6	0.12	5.7	0.20	5.9	0.45	6.7	0.25	9.0	0.00
70	22	8.3	0.26	5.7	0.19	7.7	0.34	7.2	0.18	8.7	0.18
71	28	7.3	0.24	4.9	0.17	7.5	0.38	6.6	0.21	9.0	0.00
72	20	7.2	0.25	4.8	0.27	6.2	0.46	6.1	0.27	9.0	0.00
237	12	8.7	0.13	6.7	0.19	8.3	0.24	7.9	0.14	9.0	0.00

[a] ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) germ plasm accession number.
[b] GI (gall index): 1 = no galls; 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; and 9 = >100 galls
[c] GS (gall size): 1 = no galls; 3 = very small; 5 = small; 7 = medium; and 9 = large galls.
[d] GA (galled area): 1 = no galls; 3 = 1 to 10% root area galled; 5 = 11 to 30%; 7 = 31 to 50%; and 9 = >50% root area galled
[e] DI (damage index): (GI + GS + GA)/3.
[f] EI (egg mass index): 1 = no egg masses; and 9 = >100 egg masses (same as for GI).

Selection of *H. cajani*–resistant accessions. After the roots for *H. cajani* females were examined, plants of accessions that had none were selected and transplanted into 30-cm-diameter pots containing nematode-free soil (same type as used for screening) for seed multiplication. The collected seeds were tested again as described for screening of accessions for resistance to *H. cajani,* and plants with no females were transplanted to nematode-free soil for seed production. This testing was followed for four cycles of selection.

RESULTS AND DISCUSSION

ICPW 62, an accession collected from Uttar Pradesh in northern India, was highly resistant, ICPW 60 was resistant, and ICPW 72 was moderately resistant to *R. reniformis* race A (Table 1). ICPWs 62, 69, and 70 were resistant to *H. cajani.* All the plants of ICPW 62 were resistant to *R. reniformis.* EI is a good indicator of nematode reproduction, and higher EIs are usually associated with greater root damage (10).

All the accessions were susceptible to *M. javanica* populations (Table 2). Race 1 produced bigger galls than those produced by race 3. Race 3 showed greater variability in galled area of root and gall size attributes. DI and EI of all the accessions were generally higher for race 1 than for race 3. ICPW 64 had lower damage indi-

ces (7.7 for race 1, and 5.4 for race 3) than other accessions.

ICPW 62 was identified as a promising source of multiple resistance to *R. reniformis* race A and *H. cajani*. *Cajanus platycarpus,* unlike *C. scarabaeoides,* apparently does not have genes for resistance to *M. javanica* (16). Because of considerable plant to plant variation, most probably due to genetic heterogeneity, it will be necessary to purify the promising accessions with resistance to *H. cajani* and *R. reniformis* by selecting plants with low FI and EI before using them in the breeding programs. The selected plants of ICPWs 62, 69, and 70 did not have cysts on their roots and they will be useful in breeding programs as well as in research to elucidate the mechanisms of resistance to *H. cajani*.

ACKNOWLEDGMENTS

I thank P. Remanandan, Genetic Resources Division, for providing the seed material for this study; Mohd. Mohiuddin, Peter Joseph, J. Shankaraiah, and V. Rama Krishna for technical assistance; and I. Radha for typing this manuscript.

LITERATURE CITED

1. Arnon, D. I. 1938. Microelements in culture-solution experiment with higher plants. Am. J. Bot. 25:322-325.
2. Cook, R., and Evans, K. 1987. Resistance and tolerance. Pages 179-231 in: Principles and Practices of Nematode Control in Crops. R. H. Brown and B. R. Kerry, eds. Academic Press, Sydney, Australia.
3. Dundas, I. S. 1984. Cytological investigations involving pigeonpea (*Cajanus cajan* (L.) Millsp.) and some native Australian *Atylosia* species. Ph.D. thesis, University of Queensland, Brisbane, Australia.
4. Hussey, R. S., and Barker, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Dis. Rep. 57:1025-1028.
5. Kumar, P. S. 1985. Crossability, genome relationships and inheritance studies in intergeneric hybrids of pigeonpea. Ph.D. thesis, University of Hyderabad, Andhra Pradesh, India.
6. Nene, Y. L., Sheila, V. K., and Sharma, S. B. 1989. A world list of chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* (L.) Millsp) pathology. Legumes Pathology Report 7. Int. Crops Res. Inst. Semi-Arid Tropics, Patancheru, Andhra Pradesh, India.
7. Remanandan, P. 1981. The wild gene pool of *Cajanus* at ICRISAT, present and future. Proc. Int. Worksh. Pigeonpeas. vol. 2. Int. Crops Res. Inst. Semi-Arid Tropics, Hyderabad, India.
8. Remanandan, P. 1990. Pigeonpea: Genetic resources. Pages 89-115 in: The Pigeonpea. Y. L. Nene, S. D. Hall, and V. K. Sheila, eds., C.A.B. International, Wallingford, U.K.
9. Saxena, K. B., Reddy, M. V., Singh, U., Lateef, S. S., Sharma, S. B., and Remanandan, P. 1990. Intra species variation in *Atylosia scarabaeiodes* (L.) Millsp. Euphytica 49:185-191.
10. Sharma, S. B., and Ashokkumar, P. 1991. A technique to evaluate pigeonpea for resistance to *Rotylenchulus reniformis*. Ann. Appl. Biol. 119:323-330.
11. Sharma, S. B., Ashokkumar, P., and McDonald, D. 1991. A greenhouse technique to screen pigeonpea for resistance to *Heterodera cajani*. Ann. Appl. Biol. 118:351-356.
12. Sharma, S. B., and McDonald, D. 1990. Global status of nematode problems of groundnut, pigeonpea, chickpea, sorghum and pearl millet and suggestions for future work. Crop Prot. 9:453-458.
13. Sharma, S. B., and Mohiuddin, M. 1993. Trypan blue stains egg masses of *Meloidogyne* species. Int. Pigeonpea Newsl. 17:28-29.
14. Sharma, S. B., and Nene, Y. L. 1988. Effect of *Heterodera cajani*, *Rotylenchulus reniformis* and *Hoplolaimus seinhorsti* on pigeonpea biomass. Indian J. Nematol. 18:273-278.
15. Sharma, S. B., Remanandan, P., and Jain, K. C. 1993. Resistance to cyst nematode (*Heterodera cajani*) in pigeonpea cultivars and in wild relatives of *Cajanus*. Ann. Appl. Biol. 123:824-829.
16. Sharma, S. B., Remanandan, P., and McDonald, D. 1993. Resistance to *Meloidogyne javanica* and *Rotylenchulus reniformis* in wild relatives of pigeonpea. J. Nematol. (Suppl.) 25:824-829.
17. Sharma, S. B., Smith, D. H., and McDonald, D. 1992. Nematode constraints of chickpea and pigeonpea production in the semiarid tropics. Plant Dis. 76:868-874.
18. Sharma, S. B., Smith, D. H., and McDonald, D. Host races of *Meloidogyne javanica* with preliminary evidence of groundnut race is widely distributed in India. Intl. Arachis Newsl. (In press.)
19. Stalker, H. T. 1980. Utilization of wild species for crop improvement. Adv. Agron. 33:111-147.
20. van der Maesen, L. J. G. 1990. Pigeonpea: Origin, history, evolution, and taxonomy. Pages 15-46 in: The Pigeonpea. Y. L. Nene, S. D. Hall, and V. K. Sheila, eds., C.A.B. International, Wallingford, U.K.