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Resistance to *Meloidogyne javanica* and *Rotylenchulus reniformis* in Wild Relatives of Pigeonpea[1]

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Abstract: Meloidogyne javanica and *Rotylenchulus reniformis* are important nematode pests of pigeonpea. Greenhouse evaluation of 66 accessions of 25 species of *Cajanus, Rhynchosia,* and *Flemingia* for resistance to *M. javanica* based on number and size of galls, galled area of root, and number of egg masses showed resistance to be available in these wild relatives of pigeonpea. Thirty-five accessions had ≤10 galls. Five accessions of *C. scarabaeoides* (ICPW 92, 101, 103, 128, and 133) had very small or no galls. Damage indices (based on gall number, gall size, and galled area of root) ranged between 1 and 8 on a 1 (highly resistant) to 9 (highly susceptible) scale. ICPW 92 was highly resistant to *M. javanica,* and 38 other accessions were resistant. Accessions of *Flemingia* spp. and *Rhynchosia* spp. showed greater susceptibility than accessions of *Cajanus* spp. Based on the number of egg masses on roots, no accession of the three genera was highly resistant to *R. reniformis,* and 83% of the tested accessions were susceptible. Two accessions of *C. scarabaeoides* (ICPW 38 and 92) and one accession each of *R. aurea* (ICPW 210), *R. minima* (ICPW 237), and *R. rothii* (ICPW 257) were resistant to *R. reniformis.* Species of *Cajanus* and *Flemingia* were generally more susceptible to *R. reniformis* than were *Rhynchosia* spp. ICPW 92 was identified as a promising genotype with genes for resistance to both nematodes.

Key words: Cajanus spp., *Flemingia* spp., *Meloidogyne javanica,* multiple resistance, nematode, pigeonpea, *Rotylenchulus reniformis, Rhynchosia* spp.

Cajanus cajan (pigeonpea) is the only cultivated food crop species in the Cajaninae subtribe of the economically important leguminous tribe Phaseoleae (13). The genus *Cajanus* sensu lato has 32 species (13). These, as well as species of *Flemingia* and *Rhynchosia,* are reservoirs of useful genes not available in pigeonpea germplasm, and their genetic potential in crop improvement is well demonstrated (5,6). Accessions of these species with 28–30% seed protein content (compared with 24% in the cultivated pigeonpea) have been identified; others have resistance to pod borer, podfly, or bruchids (5). The transfer of specific genes from wild species into commercial cultivars has been successful in cotton, maize, sugarcane, tobacco, and others (12). Similarly, interspecific hybridization between *Cajanus* species is possible (2,4). The root-knot nematode, *Meloidogyne javanica,* and the reniform nematode, *Rotylenchulus reniformis,* are important nematode pests of pigeonpea; they adversely affect pigeonpea growth and reduce plant biomass and grain yield in many pigeonpea growing regions (8,9,11). Our objective was to evaluate accessions of 13 *Cajanus* spp., three *Flemingia* spp., and nine *Rhynchosia* spp. for resistance to *M. javanica* and *R. reniformis.*

MATERIALS AND METHODS

Seeds of 66 accessions of 25 species of *Cajanus, Flemingia,* and *Rhynchosia* were obtained from the Genetic Resources Unit of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. All the seeds were mechanically scarified to facilitate germination. Four seeds of each accession were sown in autoclaved riverbed sand + vertisol (Typic Pellustert, silty clay loam; 39% sand, 20% silt, 41% clay; pH 8.0) mixture (4:1, v/v) in 15-cm-d pots. Eggs of a *M. javanica* population that does not reproduce on 'Florunner' groundnut were collected originally from pigeonpea, maintained on tomato (*Lycopersicon esculentum* 'Rutgers') and extracted from 8-week-old cultures by treatment with sodium hypochlorite (3). Five thousand nematode eggs in aqueous suspension were placed in the same depression in which seed were

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sown. Eight weeks after seedling emergence, pots were gently tapped to loosen the soil from around the roots. Roots were carefully washed with tap water and were evaluated for gall index, gall size, and the area of root galled (%), based on the visual assessment described below. Nematode reproduction was measured by counting egg masses. Roots were treated with 0.25% trypan blue to stain the egg masses blue (7).

Roots were rated on a 1–9 scale for gall index (GI): 1 = 0 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; 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. GI, GS, and GA are intrinsic components of damage by the root-knot nematodes and were given equal weight in assessing the damage caused by the nematode. A damage index (DI) was calculated by dividing the sum of GI, GS, and GA by three. Accessions with DI = 1 were considered highly resistant, with DI = 2–3 as resistant, with DI = 4–5 as moderately resistant, with DI = 6–7 as susceptible, and with DI = 8–9 as highly susceptible. Numbers of egg masses were rated using the same 1–9 scale used for rating gall number (1 = no egg masses, 9 = >100 egg masses).

To evaluate resistance to *R. reniformis* race A (which reproduces on castor, cowpea, and cotton), four seeds of each accession were sown in 15-cm-d pots filled with sandy clay loam soil (Udic Rhodustalf; 60% sand, 7% silt, 33% clay; pH 5.9) infested with 1,000–1,500 *R. reniformis*/100 cm^3. Within 5–6 weeks of seedling emergence, plants were gently removed from the pots, and the roots were dipped for 3 minutes in 0.25% trypan blue (7) and washed with tap water to remove excess stain. Number of egg masses per root were counted and egg mass index (EI) was rated on a 1 (highly resistant) to 9 (highly susceptible) scale: 1 = no egg masses; 2 = 1–5 egg masses; 3 = 6–10 egg masses; 4 = 11–15 egg masses; 5 = 16–20 egg masses; 6 = 21–30 egg masses; 7 = 31–40 egg masses; 8 = 41–50 egg masses; and 9 = >50 egg masses. EI is a good indicator of nematode reproduction, and higher EI is usually associated with greater root damage (7).

All accessions were evaluated in a greenhouse (maximum temperature 32 C and minimum temperature 20 C). Pots were arranged in a completely randomized design, with four pots per accession. Pots were irrigated daily with 50 ml water per pot, and quarter-strength Arnon's nutrient solution was added every week (1). Reactions of different accessions to *M. javanica* and *R. reniformis* were evaluated between 14 December 1989 and 23 March 1992. Pigeonpea genotypes ICP 7118 and ICPL 87 were used as susceptible checks for *M. javanica* and *R. reniformis*, respectively (7,11). Accessions with multiple resistance to *R. reniformis*, *M. javanica*, and to pigeonpea cyst nematode, *Heterodera cajani* (10), were identified.

Results

Thirty-nine accessions had DI ≤3 and were considered resistant to *M. javanica* (Table 1). All accessions except ICPW (ICRISAT germplasm accession number) 24, 112, 203, and 237 had small to very small galls. ICPW 92, 133, 101, and 103 had very small to no galls. Six accessions (ICPW 24, 32, 120, 112, 237, and 257) had more than 30% of root area covered with galls. ICPW 32 was the only accession with more than 30 egg masses. Sixteen accessions had between 10 and 30 egg masses. Egg masses were generally not found on ICPW 132, 133, 100, 97, 101, 128, and 106, and only 1–2 plants of these accessions had 20 or fewer egg masses. *Cajanus scarabaeoides*

TABLE 1. Evaluation of accessions of *Cajanus, Flemingia,* and *Rhynchosia* spp. for resistance to *Meloidogyne javanica* and *Rotylenchulus reniformis.*

Species	ICPW no.†	Origin (state, country)	Number of plants examined *M. javanica*	Number of plants examined *R. reniformis*	Average *M. javanica* DI‡	Average *R. reniformis* EI§
Cajanus acutifolius	5	Australia	23	35	3 (1–5)	6 (2–9)
C. albicans	24	Tamil Nadu, India	33	15	6 (3–9)	7 (4–9)
C. cajanifolius	31	Orissa, India	30	16	5 (3–7)	8 (6–9)
C. goensis	32	Kerala, India	13	35	6 (3–8)	4 (2–7)
C. grandifolius	37	Papua New Guinea	—	13	—	6 (2–9)
C. lanceolatus	38	Australia	16	19	4 (1–7)	3 (1–7)
C. lineatus	41	Tamil Nadu, India	21	17	4 (2–6)	7 (2–9)
C. mollis	52	Himachal Pradesh, India	8	—	2 (1–3)	—
C. platycarpus	66	Maharashtra, India	26	43	5 (2–8)	5 (2–9)
C. raticulatus	75	Australia	—	23	—	6 (2–9)
C. scarabaeoides	82	Maharashtra, India	23	17	3 (1–6)	7 (5–9)
C. scarabaeoides	83	Maharashtra, India	21	18	3 (1–4)	7 (2–9)
C. scarabaeoides	84	Bihar, India	30	19	3 (1–8)	8 (3–9)
C. scarabaeoides	85	Orissa, India	17	18	4 (1–6)	8 (4–9)
C. scarabaeoides	86	Karnataka, India	—	17	—	8 (4–9)
C. scarabaeoides	87	Tamil Nadu, India	27	18	2 (1–3)	8 (5–9)
C. scarabaeoides	88	Andhra Pradesh, India	27	16	2 (1–4)	7 (4–9)
C. scarabaeoides	89	Himachal Pradesh, India	17	16	3 (1–6)	8 (6–9)
C. scarabaeoides	90	Himachal Pradesh, India	16	17	2 (1–3)	7 (4–9)
C. scarabaeoides	91	Punjab, India	30	20	2 (1–4)	6 (2–9)
C. scarabaeoides	92	Himachal Pradesh, India	28	24	1 (1–2)	3 (2–7)
C. scarabaeoides	93	Sri Lanka	24	16	2 (1–3)	7 (4–9)
C. scarabaeoides	94	Sri Lanka	9	17	3 (1–4)	7 (3–9)
C. scarabaeoides	95	Myanmar	—	22	—	6 (2–9)
C. scarabaeoides	96	Uttar Pradesh, India	19	19	7 (1–3)	8 (3–9)
C. scarabaeoides	97	Uttar Pradesh, India	24	14	2 (1–3)	6 (2–9)
C. scarabaeoides	98	Uttar Pradesh, India	23	19	3 (1–6)	6 (1–9)
C. scarabaeoides	99	Uttar Pradesh, India	23	15	2 (1–3)	5 (2–8)

TABLE 1. *Continued*

Species	ICPW no.†	Origin (state, country)	Number of plants examined: *M. javanica*	Number of plants examined: *R. reniformis*	Average *M. javanica* DI‡	Average *R. reniformis* EI§
C. scarabaeoides	100	Uttar Pradesh, India	10	17	2 (1–4)	6 (1–9)
C. scarabaeoides	101	West Bengal, India	30	16	2 (1–3)	6 (1–9)
C. scarabaeoides	103	Bihar, India	16	—	2 (1–4)	—
C. scarabaeoides	105	Bihar, India	31	18	2 (1–4)	7 (1–9)
C. scarabaeoides	106	Bihar, India	12	—	2 (1–3)	—
C. scarabaeoides	109	Karnataka, India	20	17	2 (1–4)	8 (6–9)
C. scarabaeoides	110	Andhra Pradesh, India	22	16	2 (1–3)	8 (5–9)
C. scarabaeoides	111	Maharashtra, India	29	17	4 (2–6)	8 (5–9)
C. scarabaeoides	112	Maharashtra, India	10	—	6 (2–8)	—
C. scarabaeoides	115	Assam, India	28	16	2 (1–3)	6 (4–8)
C. scarabaeoides	116	Sikkim, India	18	—	2 (1–3)	—
C. scarabaeoides	117	Tamil Nadu, India	21	14	2 (1–3)	7 (6–9)
C. scarabaeoides	118	Orissa, India	25	—	2 (1–3)	—
C. scarabaeoides	119	Philippines	27	29	5 (2–7)	8 (4–9)
C. scarabaeoides	120	Philippines	24	27	5 (1–8)	7 (4–9)
C. scarabaeoides	121	Karnataka, India	20	—	2 (1–3)	—
C. scarabaeoides	122	Tamil Nadu, India	30	20	2 (1–4)	8 (5–9)
C. scarabaeoides	124	Uttar Pradesh, India	32	—	2 (1–4)	—
C. scarabaeoides	125	Tamil Nadu, India	18	—	3 (1–4)	—
C. scarabaeoides	126	Orissa, India	19	8	4 (1–6)	7 (3–9)
C. scarabaeoides	128		29	27	2 (1–4)	6 (2–9)
C. scarabaeoides	130	Andhra Pradesh, India	17	15	4 (2–5)	7 (2–9)
C. scarabaeoides	132	Orissa, India	27	13	2 (1–3)	7 (5–9)
C. scarabaeoides	133	Australia	29	15	2 (1–3)	7 (1–9)
C. sericeus	160	Maharashtra, India	20	10	5 (2–7)	7 (3–9)
C. volubilis	172	Andhra Pradesh, India	22	18	2 (1–3)	7 (5–9)
Flemingia macrophylla	194	Uttar Pradesh, India	—	6	—	7 (3–9)
F. stricta	202	Andhra Pradesh, India	28	55	5 (2–8)	6 (2–9)

TABLE 1. *Continued*

Species	ICPW no.†	Origin (state, country)	Number of plants examined *M. javanica*	Number of plants examined *R. reniformis*	Average *M. javanica* DI‡	Average *R. reniformis* EI§
F. strobilifera	203	Andhra Pradesh, India	13	17	5 (2–7)	5 (3–9)
Rhynchosia aurea	210	Andhra Pradesh, India	19	26	5 (1–8)	3 (2–5)
R. bracteata	215	Myanmar	18	17	3 (1–4)	5 (2–9)
R. cana	217	Tamil Nadu, India	18	11	3 (1–5)	6 (2–9)
R. densiflora	224	Tamil Nadu, India	26	25	3 (1–7)	5 (2–9)
R. minima	237	New Delhi, India	29	30	7 (2–9)	2 (1–2)
R. rothii	257	Maharashtra, India	34	33	5 (2–7)	2 (1–4)
R. rufescens	264	Tamil Nadu, India	27	28	4 (2–6)	7 (3–9)
R. suaveolens	265	Andhra Pradesh, India	19	15	3 (1–5)	6 (2–9)
R. sublobata	268	South Africa	—	26	—	6 (2–9)

† Accession number assigned by the International Crops Research Institute for the Semi-Arid Tropics.
‡ Damage index (DI) = (gall index (1–9 scale) + gall size (1–9 scale) + % root area galled (1–9 scale))/3. Numbers in parentheses indicate range of DI.
§ EI = Egg mass index.
— = not tested.

(ICPW 92) was highly resistant to *M. javanica* (Table 1). Accessions of *Flemingia* and *Rhynchosia* spp. were more susceptible to *M. javanica* than were accessions of *Cajanus* spp. ICP 7118 (check) was highly susceptible (DI = 8 and EI = 7). No accession of these genera was highly resistant to *R. reniformis,* but five accessions were resistant: ICPW 38, 92, 210, 224, 237, and 257 (Table 1). Large variation in EI within a given accession was observed. *Cajanus* spp. were apparently more susceptible than *Rhynchosia* spp. to *R. reniformis.* ICPW 32, 66, 99, 203, 215, and 224 were moderately resistant, and 83% of the accessions were susceptible to *R. reniformis.* Susceptible check ICPL 87 was rated 9.

DISCUSSION

This is the first attempt to explore genes for resistance to *M. javanica* and *R. reniformis* in wild relatives of pigeonpea, and the data presented are evidence of widespread resistance to *M. javanica* within the genus *Cajanus.* Some of the resistant germplasm is genetically compatible with *C. cajan* (5,13). Sixty-five percent of the accessions were resistant to *M. javanica,* 9% were resistant to *R. reniformis,* and ICPW 92 was the only accession resistant to both nematodes (Table 2). Three accessions of *C. scarabaeoides* resistant to *M. javanica* were susceptible or moderately resistant to *R. reniformis.* Reactions of these accessions suggest that genes for resistance to the two nematode species differ. Conventional breeding methods have not been successful in obtaining fertile crosses between *C. cajan* and *Flemingia* spp. or *Rhynchosia* spp., and the newer genetic techniques will be needed to transfer useful genes from these species to pigeonpea.

These studies showed that germplasm of wild *Cajanus* spp. and related genera is a source of resistance to *M. javanica* and *R. reniformis.* Some of these accessions are also promising sources of resistance to *H. cajani* (10) and have desirable attributes of early maturity and high seed protein (Table 2).

TABLE 2. Characteristics of accessions of *Cajanus* spp., *Flemingia strobilifera,* and *Rhynchosia* spp. with multiple resistance to *Meloidogyne javanica, Rotylenchulus reniformis,* and *Heterodera cajani.*

Accession	Days to flowering	Days to maturity	Seed/ pod	Seed color	Seed weight (g/100 seed)†	Seed protein content (%)	*M. javanica*	*R. reniformis*	*H. cajani*
Cajanus scarabaeoides (ICPW 92)	66	103	4.0	Grey	1.9	30.7	HR	R	MR
C. scarabaeoides (ICPW 99)	75	115	4.6	Grey	1.9	—	R	MR	S
C. scarabaeoides (ICPW 111)	59	115	4.6	Grey	2.5	25.6	MR	HS	R
C. lanceolatus (ICPW 38)	132	150	4.6	Dark	4.0	—	MR	R	MR
Flemingia strobilifera (ICPW 203)	228	254	—	Grey	1.7	—	MR	MR	R
Rhynchosia auria (ICPW 210)	52	74	1.6	Grey	2.6	—	MR	R	R
R. rothii (ICPW 257)	70	120	—	Cream	5.2	30.0	MR	R	R
R. bracteata (ICPW 215)	170	—	—	Grey	4.4	29.0	R	MR	S
R. densiflora (ICPW 224)	115	135	2.0	Grey	1.5	—	R	MR	R

HR = highly resistant; HS = highly susceptible; R = resistant; MR = moderately resistant, S = susceptible; — = not tested.
† Data from germplasm evaluation tests conducted by Genetic Resources Unit, ICRISAT.

Additional work is needed to purify the accessions before use in the intergeneric hybridization program to understand the resistance mechanisms and the genetic basis for each.

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