Ann. appl. Biol. (2000), **136**:247-252
Printed in Great Britain

Tolerance to reniform nematode (*Rotylenchulus reniformis*) race A in pigeonpea (*Cajanus cajan*) genotypes

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(Accepted 15 March 2000; Received 11 January 1999)

Summary

The reniform nematode (*Rotylenchulus reniformis*) is an important pathogen of pigeonpea (*Cajanus cajan*). Forty-six medium maturity (mature in 151-200 days at Patancheru, India) pigeonpea genotypes were evaluated for resistance and tolerance to the reniform nematode in greenhouse and field tests, over the period 1990-97. Each genotype was screened for number of nematode egg masses on a 1 (no egg mass = highly resistant) to 9 (> 50 egg masses = highly susceptible) scale. Plant biomass production in carbofuran-treated plots was compared with that in non-treated plots in a field naturally infested with *R. reniformis*. Pigeonpea genotypes C 11, ICPL 87119 and ICPL 270 were used as nematode susceptible checks. Genotypes with good plant growth, both in nematode-free and nematode-infested plots, were identified as tolerant and evaluated for plant growth and yield for at least three years. All the tested genotypes were susceptible (7 and 9 egg mass score). Single-plant-selections, based on plant vigour and yield, were made from genotypes showing tolerance to nematode infection. The level of tolerance was enhanced by plant-to-progeny row selection for plant vigour and seed yield in a nematode-sick field for at least three years. The most promising nematode tolerant genotypes produced significantly greater yield and biomass than the locally grown pigeonpea cultivars in fields naturally infested with *R. reniformis* at two locations. Pigeonpea landraces are considered to be the most likely sources of tolerance to the nematode. These reniform nematode tolerant lines represent new germplasm and they are available in the genebank of pigeonpea at ICRISAT bearing accession numbers ICP 16329, ICP 16330, ICP 16331, ICP 16332, and ICP 16333.

Key words: Landraces, pigeonpea, reniform nematode, tolerance

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is the only cultivated food crop species in the Cajaninae subtribe of the economically important leguminous tribe Phaseoleae (van der Maesen, 1990). It is widely grown by small farmers as a subsistence crop in the semi-arid tropics, particularly in India, Kenya, Myanmar, Malawi and Uganda (Nene & Sheila, 1990). It is an important source of protein in the largely cereal-based diets of people in the Indian subcontinent. The reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira, is an important pathogen of pigeonpea. It retards the growth of pigeonpea plants, and reduces plant biomass and grain yield in many pigeonpea-based production systems (Sharma & McDonald, 1990; Sharma, Smith & McDonald, 1992). The nematode causes uneven plant growth in pigeonpea on inceptisols in northern India and on alfisols in southern and western India (Sharma, Rupela & Reddy, 1993). Damage thresholds range from one to four nematodes per cubic centimeter of soil, depending on soil type and climate (Sharma & Nene, 1988; Sharma *et al.*, 1992). The roots of nematode infected plants have a "dirty root" appearance, particularly at the seedling stage, and this is a useful indicator for suspected reniform nematode infection in a field. Estimates of crop losses suggest that this nematode causes 16-19% loss in pigeonpea yield in two states in northern India (Ali, 1997). The widespread distribution of the nematode at damaging population densities in pigeonpea-based production systems is the reason for its economic importance in India, even when the most conservative estimates of the damage it causes are used. At present, there are no practical management options available to farmers to alleviate the damage caused by reniform nematode. Nematicides are too expensive to be used in subsistence agriculture, so growing nematode resistant pigeonpea cultivars is a desirable management option.

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At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, more than 8000 accessions of pigeonpea germplasm have been screened for resistance to the nematode without much success. However, wild relatives of pigeonpea have resistance to the reniform nematode (Sharma, Remanandan & McDonald, 1993; Sharma, 1995), which can be transferred to cultivated pigeonpea.

The aim of this study was to evaluate promising Fusarium-wilt resistant advanced breeding lines of pigeonpea for resistance and tolerance to the reniform nematode in greenhouse and field tests and to improve the level of nematode resistance and/or tolerance found.

Materials and Methods

Nematode population

An isolate of *R. reniformis* Race A was collected from a pigeonpea field at the research farm of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru (18°N, 78°E), Andhra Pradesh, India, and maintained on a susceptible pigeonpea cultivar ICPL (ICRISAT pigeonpea line) 87 in a greenhouse.

Greenhouse trials

Seeds of 46 medium-maturity (mature in 151-200 days at Patancheru, India) Fusarium-wilt resistant advanced breeding lines were obtained from the Pigeonpea Breeding Unit at ICRISAT (Table 1). Three seeds of each pigeonpea genotype were sown in each of five, 15 cm diameter pots filled with sandy clay loam soil (Udic Rhodustalf, 60% sand, 7% silt, 33% clay, pH 5.9) infested with eight *R. reniformis* cm^{-3}. Five to six weeks after seedling emergence, plants were removed from the pots, gently shaken free of soil, and the roots were dipped for 2 min in 0.25% trypan blue (Sharma & Ashokkumar, 1991); the roots were then washed with tap water to remove excess stain. All the egg masses were stained blue whilst roots remained unstained. Numbers of egg masses per root were counted and an egg mass index (EI) was assigned 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 values are usually associated with greater root damage. Two medium-maturity cultivars, ICPL 270 and ICPL 87119, were used as nematode susceptible checks. All the pigeonpea genotypes tested were grown in a greenhouse. Pots were watered daily with 50 ml of water per pot, and quarter-strength Arnon's nutrient solution was added every week (Arnon, 1938).

Initial identification of tolerant lines

All of the 46 medium maturity genotypes were evaluated for growth and yield in field trials in a reniform nematode-sick field. The genotypes were sown in two blocks with three replications per block; one block was treated with 4 kg a.i. carbofuran ha^{-1} and the other block acted as the untreated control. Individual plots were 4 m long by two rows wide; plant-to-plant distance was 20 cm for medium-maturity genotypes and the distance between rows was 60 cm. Seeds were sown in June and surface irrigation was applied twice during the crop growth period. At pod initiation, plant growth in all plots was examined and rated on a 1 to 5 scale: 1 = excellent uniform growth (> 90% plants showing uniform good growth); 2 = very good growth (81-90% plants showing uniform good growth); 3 = good growth (71-80% plants showing uniform good growth); 4 = moderate growth (51-70% plant showing good growth); and 5 (50% or less plants showing good growth). Plant biomass and seed yields were measured at crop maturity. Yields of each pigeonpea genotype in the two treatments were compared and genotypes with similar seed yields (± 10%) in the nematicide-treated and untreated plots were identified. Those with yields greater than the national average of 700 kg ha^{-1} were selected for further testing.

Selection for yield among tolerant genotypes

Based on the results from greenhouse and preliminary field trials, seven medium maturity lines (ICPLs 8357, 85068, 85073, 89049, 89050, 89051, and 90097) were selected for testing in further field trials. The plot size was increased to four rows of 4 m length and the genotypes were sown in a randomised complete block design with four replications. The plots were irrigated and hand-weeded twice before pod initiation. Plant growth was assessed as previously

Table 1. *List of medium maturity pigeonpea genotypes that were screened for resistance to* Rotylenchulus reniformis *(ICPL refers to accession number of ICRISAT pigeonpea lines)*

ICPL 227, 332, 335, 8357, 84060, 85061, 85062, 85063, 85065, 85066, 85067, 85068, 85069, 85070, 85071, 85073, 87090, 87120, 87121, 87122, 87123, 88043, 88044, 88045, 88046, 88047, 89042, 89043, 89044, 89045, 89046, 89047, 89048, 89049, 89050, 89051, 89052, 90096, 90097, 90098, 90099, 90100, 90101, 90102, 90103

described. The widely cultivated medium maturity pigeonpea cultivars ICP 8863, C11 and ICPL 87119 were used as checks.

In every plot, individual plants showing excellent vigour and podding were identified and single-plant selections were made for further studies. In 1993-94, seeds of each single-plant selection were sown in separate plots 4 m long by two rows wide (i.e. 40 plants). There were 20 selections from ICPL 8357, 10 each from 85068, 85073 and 89049, five from 89050, 15 from 89051, and 30 from 90097. The plots were irrigated at planting time in June and at 50% flowering. Hand weeding was done on 2 August. At the pod initiation stage, plants were rated for vigour of plant growth and pod initiation in each plot. At harvest, plant biomass and seed yield were recorded in each plot. Out of the 100 single-plant selections, 27 (12 from ICPL 90097, seven from 8357, two each from 89050, 89051, 89068, and 89073) were chosen for further testing in the 1994-95 season, using three replications but otherwise with experimental details as described previously. Crop growth was rated at the pod stage and plant biomass and seed yields were recorded at harvest in January. The seed of selections of a given pigeonpea genotype showing uniformly vigorous growth (growth score 1) was bulked, and five bulks (four of ICPL 90097 and one of 8357) were tested in 1995-96. Plot size was 4 m long by four rows wide, and there were four replications of each bulk. Data on plant growth, plant biomass and seed yield were collected.

Yield of advanced selections at two sites

In 1996-97, the five selections that were bulked for testing in 1994-95 were tested again in reniform nematode-sick soils at Dharwad (in Karnataka State in southern India) and at Patancheru in Andhra Pradesh. The soil types were a vertisol (black soil, typic Pellustert) at Dharwad, and an alfisol (red soil, Udic Rhodustalf) at Patancheru. ICP (ICRISAT pigeonpea germplasm accession) 8863, and ICPL 87119 were used as checks for comparison with the yields of selected genotypes. In addition, locally adapted and locally grown pigeonpea cultivars, Japan Super at Dharwad and C 11 at Patancheru, were included as checks for comparison of performance of the test genotypes. Each pigeonpea genotype was sown in plots consisting of four rows of 4 m length. Row spacing was 75 cm and plant spacing within rows was 25 cm. The genotypes were planted in a randomised block design with four replications. Two sprays of endosulfan were given to control insect pest attack between October and December. The plots were weeded three or four times by hand. Data on days to maturity, seed yield, dry matter yield, and nematode density in the soil were recorded.

Assessment of R. reniformis *population*

The fields selected for the trials were known to be *R. reniformis* endemic sites. Soil samples were collected to assess the nematode density at planting and harvest times every year. Four soil cores, 2.5 cm diam. and 20 cm deep, were collected from each plot, bulked, and nematodes extracted from 100 cm^3 sub-samples by wet decanting and sieving the soil suspension through 850 μm and 38 μm aperture sieves and placing the residue from the 38 μm sieve on modified Baermann funnels. The data on plant growth and nematode population densities were compared using the analysis of variance technique.

Results

Initial identification of tolerant lines

In greenhouse tests, all 46 medium-maturity pigeonpea genotypes were found to be good hosts for *R. reniformis*, with mean EI values ranging from 7 to 9.

In preliminary field trials, 32 of the 46 genotypes in Table 1 (except for ICPLs 8357, 88046, 89051, 89052, 85062, 85065, 85067, 85069, 85073, 88044, 89042, 89046, 90097, and 90099) showed marked reductions in plant biomass production in the nematode-infested soil. Nematicide application, in general, improved plant growth and seed yield, with more than 100% increase in the seed yield of 10 genotypes (ICPLs 270, 85061, 85070, 87120, 87123, 88045, 89043, 89046, 90096, and 90100) in the nematicide-treated plots. Seed yields of seven genotypes (ICPLs 88047, 87121, 88043, 89035, 89044, 89047, and 89048) increased by about 50% in the nematicide-treated plots over non-treated control plots. Seven genotypes (ICPLs 8357, 85068, 85073, 89049, 89050, 89051, 90097) which showed similar plant growth, vigour, and yield in the nematicide-treated and control plots were evaluated further.

Selection for yield among tolerant genotypes

A hundred single-plant selections were made and, eventually, only the five best selections were retained by 1995. These included four selections from ICPL 90097 and one from ICPL 8357, all of which produced good uniform plant growth and seed yield (1500 to 2500 kg ha^{-1}) in 1994-95 and 1995-96. ICPL 90097 is derived from a cross between C 11 and ICP 10958, the latter being a landrace collected from the state of Uttar Pradesh in northern India. This landrace is the most probable source of the ability to grow well in nematode infested soils as the other parent, C 11, does not grow well in reniform nematode-sick soil. ICPL 8357 is a selection from a landrace, ICP 7626, that also was collected from Uttar Pradesh. This state is a traditional pigeonpea growing region and has widespread infestations of cyst nematode (*Heterodera cajani* Koshy) and *R. reniformis* on pigeonpea (Sharma, Ali, Upadhyay & Ahmed, 1996).

Yield of advanced selections at two sites

At Patancheru, ICPL 90097-27-4-B produced significantly greater yield than the local checks, C 11 and ICPL 270. Even ICP 8863 produced about 900 kg less seed ha^{-1} than this selected genotype. The data on plant heights of five randomly selected plants per plot showed that the mean height of ICPL 90097-10-5-B was the lowest (140 cm) and of ICP 8863 the highest (203 cm), but that there was no relationship between height and grain yield (Table 2). The growth scores of the test genotypes were either 1 or 2.

At Dharwad, the performance of the test genotypes also was generally much better than that of the checks (Table 3). Selection ICPL 90097-5-5-B produced a significantly (P = 0.05) higher yield than all other genotypes except ICPL 90097-24-1-B. These two genotypes produced 600-800 kg more seed ha^{-1} than the popular cultivar ICP 8863 (Table 3). The growth of the test genotypes was uniform and good compared with the checks.

R. reniformis population assessment

In the nematode infested field at Patancheru, the nematode density at planting in the different years ranged between two and 12 nematodes cm^{-3} soil. Soil samples collected at harvest in 1995-96 revealed that the nematode density in some plots increased to 18.5 *R. reniformis* cm^{-3} soil. In the 1996-97 season, the average nematode density was 6.7 cm^{-3} soil at the time of planting in June and 9.5 cm^{-3} soil at the time of harvest in 1997. At Dharwad, the mean nematode density at the time of planting was 1.6 cm^{-3} soil and

Table 2. *Performance of medium-duration pigeonpea lines selected for tolerance to* Rotylenchulus reniformis *at Patancheru, Andhra Pradesh state, 1996-97*

Pigeonpea Line (ICPL)	Seed yield (kg ha^{-1})	Plant height (cm)	Days to mature	Nematode density cm^{-3} soil (harvest)
90097-27-4-B	2155	191	183	11.2
90097-27-1-B	1924	196	182	10.4
90097-10-5-B	1660	140	188	11.2
8357-13-4-B	1448	192	182	8.0
90097-5-5-B	1555	178	178	13.4
Checks				
ICP 8863	1256	203	181	5.6
C 11	626	187	181	9.8
ICPL 270	843	148	179	5.2
SE ±	244.6	16.1	2.7	2.5

Plant height is a mean of five randomly selected plants plot^{-1}. The mean nematode density at planting was 6.7 cm^{-3} soil.

Table 3. *Performance of medium-duration pigeonpea lines selected for tolerance to* Rotylenchulus reniformis *at Dharwad, Karnataka, 1996-97*

Pigeonpea Line (ICPL)	Seed yield (kg ha^{-1})	Days to mature	Nematode density cm^{-3} soil (harvest)
90097-27-4-B	1329	180	36.4
90097-24-1-B	1699	196	43.1
90097-10-5-B	1361	185	40.6
8357-13-4-B	1338	160	37.1
90097-5-5-B	1898	201	39.0
Checks			
ICP 8863	1111	200	40.4
Japan Super	1107	135	39.4
ICPL 87119	1241	160	39.2
SE ±	99.7	0.5	2.2

The mean nematode density at planting was 1.6 cm^{-3} soil.

39.7 cm^{-3} soil at the time of harvest in 1997. These data confirmed the high infestation levels of the reniform nematodes at the test locations (Tables 2 & 3) but that there were differences in the population build-up from planting time to harvest on the genotypes, especially those used as checks.

Discussion

This is the first attempt to identify and improve pigeonpea genotypes for tolerance to *R. reniformis*. The results show that pigeonpeas tolerant to *R. reniformis* can be selected and improved by plant-to-progeny row selection for plant vigour and yield in a nematode-sick field. We considered tolerance as ability of a genotype to produce uniformly good biomass (no stunting or yellowing) and seed yield in a reniform nematode-sick field. Visual observations on plant growth and podding, measurement of seed yield, and comparison with the local cultivars were the parameters used to decide whether or not a genotype has tolerance. The check cultivars used in this study are widely used cultivars in India and produce high yields in soils that are not infested with the reniform nematode.

The tolerant genotypes selected during this study represent new germplasm capable of producing good yields on reniform nematode infested soils. These genotypes were deposited in the pigeonpea genebank at ICRISAT and allocated the accession numbers ICP 16329 (= ICPL 90097-5-5-B), ICP 16330 (= ICPL 90097-10-5-B), ICP 16331 (= ICPL 90097-27-1-B), ICP 16332 (= ICPL 90097-27-4-B) and ICP 16333 (= ICPL 8357-13-4-B). Since these genotypes allow nematode reproduction, there is little selection pressure on the nematode population to develop highly virulent races and, as evidenced from our tests, they allow large nematode populations to build up (Table 3). Limited quantities of seed of this germplasm can be obtained on request from the Pigeonpea Genebank Curator at ICRISAT. The new germplasm may be used as parents to impart nematode tolerance, and ICP 16332 may be a good candidate for inclusion in a breeder's crossing block because it performed well at both locations. It may be useful to evaluate the new genotypes for the presence of genes that confer tolerance to nematodes using the newer tools of molecular mapping and the integrative power of quantitative trait loci analysis.

These nematode tolerant genotypes also have resistance to Fusarium wilt and produce comparatively much higher yields than the local cultivars. Our recently concluded trials with these genotypes on locations with mixed infestations of *H. cajani* (predominant population) and *R. reniformis* revealed that two lines (ICPL 90097-5-5-B and ICPL-27-4-B) perform better than the local check cultivars including ICP 8863 (data not shown here). It is reasonable to suppose that these lines may have tolerance to both species of nematode. Anand, Cook & Dale (1998) have reported that the soybean cultivar Hartwig, which was bred for resistance to the soybean cyst nematode (*Heterodera glycines* Ichinohe), is also resistant to *R. reniformis* and *Meloidogyne incognita* Kofoid & White. We anticipate that these new pigeonpea genotypes, after large scale testing and seed production, will be potential contenders for release for cultivation, particularly in southern and western India and in other countries such as Fiji and the West Indies.

Presently, efforts are not being made to develop pigeonpea cultivars that are resistant to *R. reniformis*. However, we believe that some pigeonpea cultivars might have tolerance to the reniform nematode. If during the process of selection and breeding, the pigeonpea lines were evaluated (deliberately or by chance) in a nematode-sick field, then the breeder might pick lines with ability to grow well in nematode-infested soils. As the reniform nematode occurs commonly on alfisols, vertisols and inceptisols, and pigeonpea is frequently grown on these soil types, there is a chance of inadvertent selection of pigeonpea lines with tolerance to the reniform nematode.

Tolerance of nematode damage has been found to be a useful trait in some other crops, such as potato and cotton, but it has not been exploited as much as has resistance (Cook & Evans, 1987; Cook, Robinson & Namken, 1997), particularly in subsistence agriculture. It is an important attribute in low-value crops (Trudgill, 1991) such as pigeonpea. The use of nematode tolerant cultivars to limit pigeonpea yield losses in reniform nematode infested soil is a good option since resistant cultivars are not available (Sharma *et al.*, 1995).

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