Ann. appl. Biol. (1991), **118**, 351–356
Printed in Great Britain

A greenhouse technique to screen pigeonpea for resistance to *Heterodera cajani**

By S. B. SHARMA, P. ASHOKKUMAR and D. McDONALD

Legumes Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru P.O., Andhra Pradesh 502 324, India

(*Accepted 11 December 1990*)

Summary

Heterodera cajani is an important nematode pest of pigeonpea in India and a simple and reliable greenhouse procedure has been developed to screen pigeonpea genotypes for resistance to it. In pot experiments, white cysts of *H. cajani* were counted on the roots of the susceptible genotype ICPL 87 at 15, 30 and 45 days after seedling emergence in soils infested with different levels of *H. cajani*. The seedlings were rated for the number of white cysts per root system on a one (highly resistant, no cysts) to nine (highly susceptible, more than 30 cysts) scale. White cysts were not easy to see on wet roots but were clearly visible on slightly dried roots. Cyst counts and ratings were more uniform when roots of 30 day old seedlings were evaluated than when 15 or 45 day old seedlings were examined. Effects of different *H. cajani* infestation levels on the ratings were not significant although the use of higher inoculum densities (16 to 27 eggs and juveniles/cm^3 soil) was effective in reducing variability. This procedure was used to screen 60 pigeonpea genotypes and all of them were rated seven or nine. Ten accessions of *Atylosia* spp. and *Rhynchosia* spp. were rated three.

Key words: *Atylosia* spp., *Cajanus cajan*, *Heterodera cajani*, pigeonpea cyst nematode, resistance, *Rhynchosia* spp., screening procedure

Introduction

Pigeonpea cyst nematode, *Heterodera cajani* Koshy, is the most important nematode pest of pigeonpea (*Cajanus cajan* (L.) Millsp.) in India, where more than 90% of the world's pigeonpea is cultivated (Sharma, 1988). An initial population density of three juveniles per cm^3 soil caused 25% suppression in pigeonpea (cv. ICP 2376) biomass (Sharma & Nene, 1988). Use of nematicides to control this nematode is uneconomical, and growing nematode-resistant cultivars of pigeonpea is a desirable alternative management tactic. However, limited efforts have been made to screen pigeonpea for resistance to this nematode (Velayutham, 1988) and standardised methods to screen for resistance are not available. A major difficulty in resistance screening is that *H. cajani* infection does not produce identifiable symptoms that can be used in a convenient assay. Cyst numbers on the root system can provide a useful indication of the stress imposed on a plant by nematodes, and this has been used to identify sources of resistance (Anand, 1984), but cysts of *H. cajani* are smaller in size and, therefore, more difficult to see than those of many other species of *Heterodera*. Cysts of *H. cajani* measure between 350 μm and 690 μm in length and between 210 μm and 500 μm in width while cysts of *Heterodera glycines* Ichinohe measure between 560 μm and 850 μm in length and between 350 μm and 590 μm in width (Schmitt & Noel, 1984). In a given *H. cajani* field

* Submitted as Journal Article No. 1071 by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru P.O., Andhra Pradesh 502 324, India.

population, around 50% of the cysts measure less than 500 μm in length and less than 320 μm in width (Sharma & Swarup, 1984). The objective of this study was to develop a simple and reliable greenhouse screening procedure based on numbers of white cysts on the root system to evaluate pigeonpea genotypes and to identify resistance sources for use in breeding programmes.

Materials and Methods

Production of soils with different infestation levels of Heterodera cajani

Soil infested with *Heterodera cajani* was collected in December 1986 from a Vertisol (black soil) field on which pigeonpea had been cultivated (June-February) every year for the last 15 yr at the research farm (17°N, 78°E, 545 m) of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. The nematode population extracted from the field soil was inoculated onto a susceptible pigeonpea cultivar, ICPL 87, in 20 cm diameter plastic pots containing autoclaved sand/black soil mixture (3:1) in a greenhouse maintained at approximately 30 °C. Plants were harvested after three months in March 1987 and the infested soil containing the *H. cajani* population was mixed with an equal amount of autoclaved sand/black soil (3:1). This nematode-infested soil was used to fill fifty 20 cm diameter plastic pots. Ten seeds of ICPL 87 were sown in each pot to increase the nematode inoculum further.

The nematode-infested soil produced in 20 cm diameter pots was bulked, thoroughly mixed to distribute the nematode population uniformly, and divided into three parts. The first part was maintained as such, and the other two parts were mixed with different quantities of autoclaved sand/black soil mixture (3:1) to obtain three infestation levels. Population densities of *H. cajani* at the three infestation levels were estimated at 4, 6 and 17 eggs and juveniles of *H. cajani*/cm^3 soil, using the technique described below. The three soils were used to fill 12·5 cm diameter plastic pots. Holes in the bottoms of these pots were closed with cotton wool plugs before adding 1000 cm^3 of soil to each pot.

Estimation of Heterodera cajani *infestation levels in soil*

Cysts, eggs and second-stage juveniles of *H. cajani* were extracted from 100 cm^3 soil samples by suspending them in water, pouring them through nested sieves (850, 180 and 38 μm pore sizes), and incubating the residue from the 38 μm pore sieve on double layers of Kimwipes ® tissue papers supported on a wire mesh in 9 cm diameter Petri dishes containing sufficient water to cover the residue (Schindler, 1961). The Petri dishes were incubated at 25 °C ± 2 °C for 48 h to allow the juveniles to pass from the residue to the water in the Petri dishes. Cysts were collected on the 180 μm pore sieve (Sharma & Nene, 1986). Eggs and juveniles in the cysts were counted by gently crushing 50 cysts on a glass slide. Second-stage juveniles collected in the 9 cm diameter Petri dishes were counted separately and, together with eggs and juveniles counted from cysts, gave an estimate of the total population of eggs and juveniles.

Effects of different infestation levels and plant age on white cyst counts

Seeds of ICPL 87 were sown in the pots containing soils with four, six and 17 individuals of *H. cajani*/cm^3 soil. There were 12 pots, each sown with six seeds, for each of the three infestation levels. Pots were arranged in a split plot design with dates of observations of the root systems for *H. cajani* white cysts as the main treatment and nematode infestation levels as sub-treatments. At 15, 30 and 45 days after emergence, the pots were tilted downwards at about 45° and tapped to loosen the soil from around the roots. Roots were gently washed with

tap water and numbers of white cysts were counted. Initially a hand lens was used to locate the cysts on the roots. Before counting the cysts on a root system, roots of 25 *H. cajani*-infected seedlings were examined to train the eye in locating the white cysts. Results of naked eye or hand lens counts were confirmed using a stereomicroscope (10x).

Heterodera cajani *white cysts index (WCI)*

Seedlings were rated on a one (highly resistant) to nine (highly susceptible) white cyst index (WCI) on the basis of the number of white cysts on their roots: 1 = no white cysts, 3 = 1 5, 5 = 6 - 10, 7 = 11 - 30, and 9 = more than 30 white cysts. The WCI is an indicator of nematode reproduction and of the damage to the plant: a higher WCI is associated with greater damage.

For each infestation level and time of screening, roots of 24 seedlings were evaluated and the percentages of seedlings rated as 1, 3, 5, 7 or 9 were calculated. These experiments were repeated in August-September 1988 and the results were further confirmed in experiments conducted between April 1989 and October 1989. The data on white cysts counts were statistically analysed for variance, as was the relationship between the visual cyst count and cyst count with aid of a stereomicroscope. Means, standard errors, coefficients of variation and t-values were calculated to examine the variability in the ratings of root systems.

Reaction of pigeonpea genotypes and related wild pigeonpea species

Sixty pigeonpea genotypes and 20 accessions of *Atylosia albicans, A. lanceolata, A. platycarpa, A. scarabaeoides, A. sericea, A. volubilis, Rhynchosia aurea, R. minima, R. rothii, R. rufescens* and *R. sublobata* were evaluated for their reaction to *H. cajani* using the inoculation technique and rating procedure described above. Information about province and country of collection of wild pigeonpea species was taken from Remanandan, Sastry & Mengesha (1988).

Results

Visual observations of white cysts

White cysts were not easily visible on wet roots, but slight drying of the root system, to an extent that when touched the roots did not wet the fingers, enabled the white cysts attached to the roots to be clearly seen. Holding the seedlings at an angle with the roots facing upwards further aided in recording the cyst number. Visual WCI's per root system of 30 and 45 day old seedlings were similar to the WCI's obtained using a stereomicroscope ($r = 0.74^{**}$).

Effect of plant age on WCI

Visual WCI of 15 day old seedlings was significantly ($P = 0.01$) lower than WCI of 30 and 45 day old plants, and 42 - 71% of the total seedlings scored had ratings of 3 or less. When the root systems of 30 day old seedlings were examined, 96 - 100% were rated as 9 and 18 - 52% of the seedlings were rated 9 when the roots were checked 45 days after seedling emergence.

Effect of Heterodera cajani *infestation levels on WCI*

Counts on numbers of white cysts 15 days after seedling emergence were variable and ratings were low, irrespective of the infestation levels of *H. cajani* (Table 1). Examination of root systems 30 days after emergence gave scores consistently close to 9. WCI's were lower at all three infestation levels when roots were scored 45 days rather than 30 days after seedling emergence. Similar results were obtained in the repeat experiments in 1988 and in 1989 when seeds of ICPL 87 were sown in *H. cajani* infested soil having inoculum densities of 16 and more eggs and juveniles/cm^3 soil. The mean scores 30 days after seedling emergence ranged

Table 1. *Effect of* H. cajani *infestation level and time of scoring on the resistance ratings (white cyst indices) of pigeonpea (ICPL 87). Indices are means of 24 replicates*

Time of scoring (Days after seedling emergence)	White cyst index of ICPL 87 at infestation levels of		
	4 eggs and juveniles/ cm^3 soil	6 egg and juveniles/ cm^3 soil	17 eggs and juveniles cm^3 soil
15	4.0 ± 0.24 (3-7)[1]	3.6 ± 0.25 (1-7)	4.6 ± 0.32 (3-7)
30	8.9 ± 0.08 (7-9)	8.9 ± 0.08 (7-9)	9.0 ± 0.00 (9)
45	7.9 ± 0.24 (5-9)	7.7 ± 0.20 (7-9)	7.2 ± 0.20 (5-9)

[1] Range of white cyst index

Table 2. *Reaction of wild relatives of pigeonpea to* H. cajani

ICPW No.[1]	Species	Country of origin	Province	No. of seedlings observed	WCI
24	*Atylosia albicans*	India	Tamil Nadu	10	5
38	*A. lanceolata*	Australia	Northern Territory	17	3
66	*A. platycarpa*	India	Maharashtra	22	3
89	*A. scarabaeoides*	India	Himachal Pradesh	22	3
92	*A. scarabaeoides*	India	Himachal Pradesh	20	3
94	*A. scarabaeoides*	Sri Lanka		20	3
95	*A. scarabaeoides*	Burma		10	5
96	*A. scarabaeoides*	India	Uttar Pradesh	24	3
111	*A. scarabaeoides*	India	Bihar	13	3
115	*A. scarabaeoides*	India	Assam	17	3
116	*A. scarabaeoides*	India	Sikkim	11	5
117	*A. scarabaeoides*	India	Tamil Nadu	10	5
119	*A. scarabaeoides*	Philippines	Nueva Vizeaya	21	5
160	*A. sericea*	India	Maharashtra	12	7
172	*A. volubilis*	India	Andhra Pradesh	10	5
210	*Rhynchosia aurea*	India	Andhra Pradesh	12	3
237	*R. minima*	India	New Delhi	14	7
256	*R. rothii*	India	Maharashtra	35	3
264	*R. rufescens*	India	Tamil Nadu	11	5
268	*R. sublobata*	South Africa		15	9
	Cajanus cajan			88	9

[1] ICRISAT germplasm accession number

between 8.3 and 9.0 and these scores did not differ significantly ($P = 0.01$) from the rating of 9.

Reactions of pigeonpea genotypes and related wild species

WCI's of all the 60 pigeonpea genotypes tested were 7 or 9. ICP 562, 592, 1185, 8858, 8864, 9879, 9880, 11287, 11291, 11294, 11299, 12726, 12727, 12729, ICPL 84045, 85010, 86015, 86018, 86029, 86030, 87097, ICPH 149 and 328 were rated as 7. WCI's of some accessions of *A. scarabaeoides, A. lanceolata, A. platycarpa, R. aurea* and *R. rothii* ranged from 3 to 7 (Table 2).

Discussion

Pigeonpea genotypes can be evaluated for resistance to *H. cajani* by examination of the infected roots of 30 day old seedlings for white cysts. At this stage, the seedlings usually have four trifoliate leaves and this is a good indicator as to when the seedlings should be uprooted for evaluation. The life cycle of *H. cajani* is short, and white cysts are produced 11 days after inoculation of second-stage juveniles on pigeonpea (Koshy & Swarup, 1971). Evaluation of the root system of 15 day old seedlings is easy as the root volume is small but scoring is not reliable even at an infestation level as high as 17 eggs and juveniles/cm^3 soil. The root volumes of 45 day old seedlings were large and their examination more time consuming than that of 15 and 30 day old seedlings. Many cysts had turned brown and some had dislodged from the roots. The roots had also become light brown, so reducing the contrast between the cysts and roots. This may be the reason for the low numbers of *H. cajani* cysts per root system recorded on 48 day old pigeonpea genotypes by Velayutham (1988). Precision of ratings was improved when higher initial infestation levels were used and this was reflected in lower coefficients of variation. A low inoculum density of 400 juveniles/pot, as was used by Velayutham (1988), would lead to more variable results.

This screening procedure has the advantage that genotype evaluation can be accomplished in the greenhouse continuously throughout the year. It is simple and does not involve separate inoculation of the the nematodes into each pot. Temperatures during the screening period can greatly influence the reaction of pigeonpea as the nematode takes a longer time to complete its life cycle at lower temperatures (Koshy & Swarup, 1971). Apparently, photoperiod does not affect the reaction and WCI of ICPL 87, as plants tested in different months did not vary. To determine the viability of inoculum we suggest inclusion of a susceptible check (e.g. ICPL 87) along with the test genotypes. Care is needed to avoid build up of parasites which reduce the population levels of *H. cajani*, particularly of *Pasteuria penetrans*, whose endospores may be attached to the cuticles of juveniles. Infected cysts may be filled with bacterial spores.

All the pigeonpea genotypes tested were susceptible, but some (WCI = 7) supported fewer nematodes than ICPL 87 whose cultivation in India is increasing fast. There is a need to screen a large number of pigeonpea accessions to identify resistance sources and use them in breeding programmes. Genotypes of wild pigeonpea species differ in their reactions to *H. cajani* and promising accessions of *A. scarabaeoides* can easily be crossed with pigeonpea to utilise the useful genes (Saxena *et al.*, 1990; Sharma & Nene, 1985).

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(*Received 29 October 1990*)