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Tolerance of chickpea (*Cicer arietinum* L.) lines to root-knot nematode, *Meloidogyne javanica* (Treub) Chitwood

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Abstract

The root-knot nematode, *Meloidogyne javanica* (Treub) Chitwood is an important parasite of chickpea (*Cicer arietinum* L.). Four chickpea genotypes were evaluated for tolerance to *M. javanica* in naturally infested fields at three locations. Each genotype was evaluated for number of galls, gall size, root area covered with galls and number of egg masses produced. All the cultivars were susceptible or highly susceptible. Seed yield, weight of 100 undamaged seeds, total dry matter and plant height were compared with checks. Chickpea cultivar Annigeri and a local check were used as nematode susceptible checks in all locations. The four promising nematode tolerant genotypes produced significantly greater yield and total dry matter than the checks in fields naturally infested with *M. javanica* at three locations. These *M. javanica* tolerant lines represent new germplasm and they are available in the chickpea genebank at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) bearing the identification numbers ICC 8932, ICC 11152, ICCV 90043 and ICCC 42.

Introduction

Chickpea (*Cicer arietinum* L.) is the second most important pulse crop in the world after beans (*Phaseolus vulgaris* L.) (FAO, 2000). It is grown in 33 countries and is a significant component of cropping systems of subsistence farmers in the Indian subcontinent, West Asia and North Africa. Many species of plant-parasitic nematodes have been found associated with chickpea in seventeen countries and they cause an estimated 13.7% annual loss (Sasser 1987; Nene et al. 1989). The root-knot nematodes *Meloidogyne javanica* (Treub) Chitwood and *M. incognita* (Kofoid and White) Chitwood are the most prominent nematode pests of chickpea in the tropics (Sharma and McDonald 1990). These nematodes cause galling of the roots and aerial parts of the plants manifest reduced vigor, stunting and early senescence (Sharma et al. 1992). Estimates of crop losses suggest that this nematode causes 22–84% loss in chickpea yield in two states of northern India (Ali 1997). These economic losses would be much greater if certain management practices were not employed. No chickpea cultivar has been developed with resistance to *Meloidogyne* spp. Currently, the most effective strategies for managing *M. javanica* in chickpea include the use of nematicides and rotation with non-host crops. However, nematicides are too expensive to be used in subsistence farming systems, so growing nematode resistant chickpea cultivars is a desirable management option.

At the International Crops Research Institute for the

Semi-Arid Tropics (ICRISAT), Patancheru, India, more than 7000 accessions of chickpea germplasm have been screened for resistance to the nematode without much success. The objectives of this research were to: (i) evaluate selected chickpea genotypes for tolerance to *M. javanica* (ii) measure chickpea yield suppression in presence of the nematode in the field, and (iii) determine if selected genotypes have levels of tolerance in infested field in different agro-ecological regions of India.

Materials and Methods

Based on the results from greenhouse and preliminary field trials (Sharma et al. 1995), four chickpea genotypes (ICC 8932, ICC 11152, ICCC 42 and ICCV 90043) were selected for further field trials in different agro-ecological regions. Field experiments were conducted in *M. javanica* infested fields at three locations at Anand and Derol Gujarat State in Western India and at Rahuri in Maharashtra. The widely cultivated chickpea cultivar Annigeri was used as a check for comparison with the yields of selected genotypes. In addition, locally adapted and locally grown chickpea cultivars (Dahod Yellow at Anand and Derol locations; Vishal at Rahuri) were also included as checks. Each chickpea genotype was sown in plots consisting of three rows of 4-m length. Row spacing was 30 cm and plant spacing within rows was 10 cm. The genotypes were sown in a randomized block design with three replications. The plots were irrigated and hand-weeded twice before pod initiation. Data on day to maturity, plant count at harvest, seed yield, weight of 100 undamaged seeds, total dry matter yield, plant height, nematode density, galls and egg masses, size of galls, extend of galled area of root were recorded.

Seven to eight weeks after seedling emergence, five plants were randomly taken from each plot, roots were carefully washed with tap water and evaluated for gall index, gall size and percent galled area of root. Nematode reproduction was measured by counting egg masses. Plant roots were treated with 0.25% trypan blue to stain the egg masses (Sharma and Mohiuddin 1993a). Roots were rated on a 1–9 scale for gall index (GI): 1 = 0 galls; 2 = 1–5; 3 = 6–10; 4 = 11–20; 5 = 21–30; 6 = 31–50; 7 = 51–70; 8 = 71–100 and 9 = > 100 galls. Gall size (GS) was evaluated on a 1–9 scale (1 = no galls; 3 = small galls; 5 = medium; 7 = large, and 9 = very large gall). Percent galled area (GA): 1 = no galls; 3 = 1–10% root area galled; 5 = 11–30%; 7 = 31–50%; and 9 = >50% root area galled. Number of egg masses (EI) was rated using the scale developed for gall index. To assess the root damage, a damage index (DI) was calculated by dividing the sum of GI, GS, and GA by three. DI of a plant is an indicator of its degree of susceptibility (or resistance) to root damage by the nematode. Cultivars with DI = 1 were considered highly resistance to damage, with DI = 2–3 as resistance, with DI = 4–5 as moderately resistance to damage, with DI = 6–7 as susceptible, and with DI = 8–9 as highly susceptible to damage (Sharma et al. 1993b). EI of a plant is an indicator of its suitability to nematode reproduction. Greater EI usually but not always results in greater DI. Abundant nematode reproduction and (or) severe root damage correspond to susceptibility. Tolerance is the ability of a plant to grow without any perceptible reduction in plant growth and yield despite severe root damage and (or) abundant reproduction of the nematode. Dry shoot weight and number of pods/plant were recorded from different plots.

Analysis of variance was performed to compare the means based on the arcsine-transformed data. When the effects were found to be significant, were seperate means in homogeneous groups using Duncan's Multiple Range Test ($P = 0.05$). We also computed for the interaction effects, the mean values and their respective standard errors. All the statistical analysis was done using (SAS, 1990).

Results

Tolerance

At Anand, plant growth was highly variable and roots of all genotypes were heavily galled due to nematode infection. Plants were chlorotic and several plants almost died before reaching physiological maturity. Significant differences were found in seed yield/ha ($F = 9.81$; $df = 5, 12$; $P < 0.006$), weight of 100 undamaged seeds ($F = 13.38$; $df = 5, 12$; $P = < 0.001$), total dry matter ($F = 7.45$; $df = 5, 12$; $P = < 0.022$), and plant height ($F = 4.10$; $df = 5, 12$; $P = < 0.0210$) between genotypes and checks. All genotypes produced higher yield than cultivar Annigeri and local checks (Table 1). The data on plant heights showed the mean height of all genotypes were more than that of Annigeri and the local checks. Plant heights of the check were reduced by nematode infection. Ratio of total dry matter in genotypes and checks revealed that ICC 8932, ICC 11152, ICCV 90043, and ICCC 42 were relatively tolerant.

Significant differences were found in seed yield/ha ($F = 18.82$; $df = 5, 12$; $P = < 0.001$), total dry matter ($F = 4.70$; $df = 5, 12$; $P = < 0.0132$) and plant height ($F = 5.87$; $df = 5, 12$; $P = < 0.057$) at Derol between genotypes and checks (Table 1). However, there was no difference in weight of 100 undamaged seeds between genotypes and checks. All the tested genotypes produced similar level of seed yield but more than that of the local checks. Total dry matter and plant height of checks were less than the tested genotypes.

At Rahuri, significant differences in seed yield/ha ($F = 8.36$; $df = 5, 12$; $P = <0.013$), weight of 100 undamaged seeds ($F = 11.39$; $df = 5, 12$; $P = < 0.003$) and total dry matter ($F = 6.12$; $df = 5, 12$; $P = < 0.048$) between genotypes and checks were observed. There was no difference found in plant height between tested genotypes and checks. All the genotypes produced significantly larger yield than the checks. Cultivar Annigeri and the local checks produced about 600 kg less seed/ha than the genotypes (Table 1). The ratio of total dry matter of tested genotypes was higher than that of checks.

Comparison of cultivars

We found significant differences in seed yield/ha, total dry matter, weight of 100 undamaged seeds and plant height between genotypes and checks, but there was no significant difference within genotypes (Table 2). The data on plant height showed that the mean height of all the genotypes was higher than checks.

Locations

Seed yield/ha of the tested genotypes was significantly different in three locations ($F = 2.51$; $df = 2$; $P = < 0.019$). At Rahuri and Derol, the tested genotypes produced significantly higher yield than at Anand. The data on plant heights showed that mean height at Rahuri was the highest (76.3 cm) and at Anand and Derol were almost the same (51.7 cm), but that there was no relationship between height and grain yield (Table 3).

Table 1. Performance of chickpea lines selected for tolerance to *Meloidogyne javanica* at three nematode infested sites in India.

Chickpea line	Seed yield (kg ha^{-1})	Total dry matter (kg plot^{-1})	Weight 100 seed (g)	Plant height (cm)	Nematode denisty per 100 g soil (at harvest)
Anand site					
ICC 8932	1510.2 ± 32.5b	2.5 ± 0.2a	25.8 ± 1.4a	55.4 ± 1.2a	500.0 ± 29.0abc
ICCV 90043	1671.3 ± 154.7ab	2.9 ± 0.2a	22.5 ± 1.5a	52.3 ± 2.6ab	413.0 ± 18.6c
ICC 11152	1921.3 ± 129.0a	2.7 ± 0.2a	25.1 ± 0.3a	55.0 ± 1.6a	503.0 ± 31.8ab
Annigeri	1070.4 ± 117.3c	1.9 ± 0.2b	17.0 ± 0.2b	45.8 ± 1.7c	550.0 ± 28.9a
ICCC 42	1588.0 ± 72.0b	2.7 ± 0.0a	22.8 ± 1.6a	51.0 ± 1.6abc	453.0 ± 31.8bc
Dahod yellow*	1166.7 ± 42.4c	1.9 ± 0.1b	17.5 ± 0.7b	47.4 ± 2.5c	520.0 ± 17.3ab
Derol site					
ICC 8932	1779.6 ± 46.5a	1.9 ± 0.1a	24.0 ± 2.2ab	55.0 ± 2.5a	477.0 ± 21.9a
ICCV 90043	1949.1 ± 72.8a	1.8 ± 0.2a	25.5 ± 0.6ab	54.4 ± 0.6a	517.0 ± 33.3a
ICC 11152	1992.6 ± 54.7a	1.8 ± 0.2a	26.4 ± 0.6a	52.3 ± 1.1ab	430.0 ± 20.0ab
Annigeri	1294.5 ± 77.2a	1.2 ± 0.2bc	19.0 ± 3.9b	47.6 ± 1.6bc	337.0 ± 41.8b
ICCC 42	1813.9 ± 86.1a	1.7 ± 0.0ab	25.2 ± 0.9ab	54.8 ± 0.7a	477.0 ± 76.2a
Dahod yellow*	1280.6 ± 91.9b	1.1 ± 0.2c	19.5 ± 2.4ab	46.3 ± 2.0c	477.0 ± 21.9a
Rahuri site					
ICC 8932	1583.3 ± 84.8bc	1.3 ± 0.1a	32.4 ± 1.0a	78.7 ± 0.7ab	553.0 ± 37.6a
ICCV 90043	1907.4 ± 51.5a	1.3 ± 0.0a	25.8 ± 0.8bc	80.0 ± 7.8ab	606.7 ± 53.6a
ICC 11152	1935.2 ± 106.8a	1.3 ± 0.1a	27.0 ± 0.8b	83.7 ± 1.7a	517.0 ± 38.4a
Annigeri	1287.0 ± 129.6c	0.8 ± 0.0b	23.0 ± 1.6c	68.5 ± 4.0b	483.0 ± 16.7a
ICCC 42	1722.2 ± 112.3ab	1.2 ± 0.2a	28.0 ± 1.4b	78.0 ± 1.9ab	493.0 ± 31.8a
Vishal*	1347.2 ± 60.5c	0.8 ± 0.0b	21.0 ± 1.2c	69.1 ± 1.9b	516.0 ± 44.0a

*= Local checks. Each value is the mean of three plots. Plant height is a mean of five randomly selected plants/plot. The mean nematode density at planting was 271 (Anand), 193 (Derol) and 276 (Rahuri) per 100 g of soil. Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P = 0.05$).

Table 2. Performance of chickpea lines in *Meloidogyne javanica* infested fields across locations (Anand, Deoral and Rahuri).

Chickpea line	Seed yield (kg ha^{-1})	Total dry matter (kg $plot^{-1}$)	Weight 100 seed (g)	Plant height (cm)	Nematode density per 100 g soil (at harvest)
ICC 8932	1624.4 ± 49.9c	1.9 ± 0.2a	27.4 ± 1.5a	63.0 ± 4.0a	510.0 ± 18.9a
ICCV 90043	1843.6 ± 67.3ab	2.0 ± 0.3a	24.2 ± 0.7b	62.2 ± 5.0a	512.2 ± 33.8a
ICC 11152	1949.7 ± 52.0a	1.9 ± 0.2a	26.2 ± 0.4ab	63.7 ± 5.0a	483.3 ± 20.5a
Annigeri	1217.3 ± 66.3d	1.3 ± 0.2b	19.5 ± 1.5c	54.0 ± 3.9b	456.7 ± 35.0a
ICCC 42	1708.0 ± 56.3bc	1.9 ± 0.2a	25.4 ± 1.0ab	61.3 ± 4.3a	474.4 ± 26.2a
Local check	1264.8 ± 43.0d	1.3 ± 0.2b	19.5 ± 1.0c	54.3 ± 3.9b	504.4 ± 16.6a

Each value is the mean of three plots. Plant height is a mean of five randomly selected plants/plots. The mean nematode density across locations at planting was 193 per 100 g of soil. Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P = 0.05$).

M. javanica population density

In the nematode infested fields at Anand, Rahuri and Derol the average nematode density at planting was 271, 276 and 193 per 100 g of soil. Soil samples collected at harvest in 1997 revealed that the average nematode density increased to 490 (Anand) 528 (Rahuri) and 452 (Derol) per 100 g of soil. These data confirmed the high infestation levels of *M. javanica* nematodes at the test locations.

Discussion

Confirming the finding of Sharma et al. (1995), results of this study showed that nematode tolerance in the chickpea genotypes was stable across investigated regions. All of the genotypes produced significantly higher yield compared to the standard Annigeri and local check in *M. javanica* infested field in three locations. We considered tolerance as the ability of a genotype to produce uniform good biomass and seed yield in a nematode sick field. Visual observations on plant growth, podding seed yield per ha and comparison with the local cultivars were the parameters used to decide whether a genotype has tolerance. The local check cultivars used in this study are widely used cultivars in their respective region and produce high yield in soils that are not infested with nematodes.

Four tolerance genotypes selected during these field experiments represent germplasm capable of producing good yield in *M. javanica* infested soils. Since these genotypes allow nematode reproduction, there is little selection pressure on the nematode population to develop highly virulent races and, as evident from our tests, they allow large nematode populations to build up (Table 1). Limited quantities of seed of this germplasm can be obtained on request from the chickpea genebank curator at ICRISAT. The germplasm may be used as parents to transfer nematode tolerance and ICC 11152 may be a good candidate for incorporation in a breeder's crossing block because it performed well at all locations. It may be useful to evaluate the genotypes for the presence of gene(s) that confer tolerance to *M. javanica* using the newer tools of molecular mapping and the quantitative attribute loci analysis.

At present, efforts are not being made to develop chickpea cultivars that are resistant to *M. javanica*. However, we understand during our routine screening test in a glasshouse that some chickpea cultivars might have tolerance to the root-knot nematode. If during the process of selection and breeding, the chickpea lines were evaluated (intentionally or by chance) in a nematode-sick field, then the breeder might choose lines with ability to grow well in

Table 3. Performance of chickpea lines at three locations.

Location	Seed yield (kg ha^{-1})	Total dry matter (kg $plot^{-1}$)	Weight 100 seed (g)	Plant height (cm)	Nematode density per 100 g soil (at harvest)
Anand	1488.0 ± 78.8b	2.18 ± 0.9b	24 ± 0.1a	51.2 ± 1.0b	490.0 ± 14.2ab
Derol	1685.0 ± 74.8a	2.32 ± 1.0b	16 ± 0.0b	51.7 ± 1.0b	452.2 ± 19.8b
Rahuri	1630.4 ± 69.1a	2.62 ± 1.0a	11 ± 0.0c	76.3 ± 1.9a	528.3 ± 16.7a

Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P = 0.05$).

nematode infested soils. As the root-knot nematodes are the most prominent nematode pests of chickpea in the tropics (Sharma and McDonald 1990) and chickpea is frequently grown in these areas, there is a good chance of inadvertent selection of chickpea lines with tolerance to the root-knot nematode.

Tolerance to nematode damage has been found to be a useful trait in some other crops such as pigeonpea (Sharma et al. 2000), chickpea (Sharma et al. 1995) and cotton (Koenning et al. 2000), but it has not been exploited as much as has resistance (Cook et al. 1997), especially in sustainable agriculture. It is an important characteristic in low-value crops (Trudgill 1991). The use of nematode tolerant cultivars to limit chickpea yield losses in root-knot nematode infested soil is a feasible alternative especially in the absence of nematode resistant cultivars.

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