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Screening of field pea (*Pisum sativum*) selections for their reactions to root-knot nematode (*Meloidogyne incognita*)

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Abstract: Pot studies were carried out to evaluate the reactions of 23 selections of field pea (*Pisum sativum*) against *Meloidogyne incognita* (2000 freshly hatched juveniles (J2)/pot). Experiment was conducted under greenhouse conditions ((24.7±3) °C and (62±7)% RH) and terminated 45 d after inoculation. The roots of all the selections were assessed to determine root-knot indices (RKI) on a 0~4 scale. Out of 23 selections HFP-990713, Pant P-25, and HFP-0129 were resistant; Pant P-2005, NDP-2 and Pant P-42 were tolerant; LFP-305, HFP-8909, HFP-4, HUP-31, HFP-0128, Pant P-31, Pant P-40, LFP-363, and HFP-0118 were moderately resistant; HFP-0110, HUDP-28, HUDP-15, HUDP-27, HUP-30, HUP-2 and HUDP-26 were moderately susceptible; and only Ambika was susceptible to *M. incognita*. It was observed that reproduction of nematode was favored on tolerant and susceptible cultivars but inhibited on resistant ones. Strong negative correlation was observed between the total fresh and dry plant weights and the root-knot index. The selection Pant P-42 showed highest tolerance among all the selections tested and can be recommended for field trials, whereas, selection Ambika showed highest susceptibility and should be avoided.

Key words: *Pisum sativum*, *Meloidogyne incognita*, Resistance
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INTRODUCTION

Fieldpea (*Pisum sativum* L.) is one of the important pulse crops of the world and is cultivated over 5.9 million hectares with a production of about 11.7 million tons (Singh, 1983). In India it is grown over 0.7 million hectares yielding about 0.6 million tons (Singh, 1983). Among various obstacles in cultivating this crop, root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood has been reported to cause severe yield losses of up to 20%~33% (Upadhyay and Dwivedi, 1987). *Meloidogyne* spp. are sedentary endoparasites and among the most damaging agricultural pests. The infection starts with root penetration of second stage juveniles (J2) hatched in soil from eggs encapsulated in egg masses laid by the females on the infected roots. Metabolically active giant cells are induced and maintained in susceptible hosts only by the nematode feeding. The juvenile develops into globose adult female whose eggs are deposited in a gelatinous matrix on the surface of a galled root. This intimate relationship between the root-knot nematode and host is genetically regulated in both organisms and has resulted in the evolution of resistance genes in many crop species (Sidhu and Webster, 1981). Resistance is an effective management tool that improves crop yield in the presence of nematode population densities that exceed the damage threshold. Because resistance to nematodes is usually developed by selection of plants with reduced rates of nematode reproduction, nematode population densities are typically lower following a resistant cultivar than a susceptible cultivar. However, this is not always the case if the crop has only partial resistance.

Niblack *et al*.(1986) demonstrated that at moderate to high initial population densities, *M. incognita* reach their maximum levels at about 90 d after planting on a susceptible soybean cultivar (presumably due to extensive damage to the host), whereas on partially resistant cultivars that were less severely damaged by the nematodes, the population densities were still increasing at 120 d after planting. Even though resistance to root-knot nematodes is available in several crop species including *P. sativum* (Verma and Gupta, 1993), new sources of resistance are needed to improve the level of root-knot resistance.

The current study was undertaken to evaluate the resistance of different field pea (*P. sativum*) cultivars to root-knot nematode (*M. incognita*) in pot experiments under greenhouse conditions.

MATERIALS AND METHODS

Plant material

Twenty-three cultivars (Pant P-2005, HFP-990713, Pant P-25, NDP-2, HFP-0129, Pant P-42, LFP-305, HFP-8909, HFP-4, HUP-31, HFP-0128, Pant P-31, Pant P-40, LFP-363, HFP-0118, HFP-0110, HUDP-28, HUDP-15, HUDP-27, HUP-30, HUP-2, HUDP-26 and Ambika) of *P. sativum* obtained from Indian Institute of Pulses Research (IIPR), Kanpur, India were evaluated for their reaction to *M. incognita* after artificial inoculation under controlled conditions.

Preparation of *M. incognita* inoculum

A single egg mass of *M. incognita* picked by hand with a fine forceps from an infected eggplant root was surface sterilized in 1:500 (*V*/*V*) aqueous solution of "chlorax" (Sodium hypochlorite) for 5 min, and was then transferred to a small coarse sieve lined with tissue paper to cover the bottom of the sieve that was within a petriplate containing sufficient amount of water. The petriplate was incubated at room temperature ((27±5) °C) for 5 d (den Ouden, 1958). Seedlings of eggplant grown in autoclaved soil were inoculated with the progeny of the single egg mass in order to get regular supply of the inoculum for the experiment.

Experimental procedure

Experiment was conducted in a greenhouse ((24.7±3) °C, (62±7)% RH) in 138 earthen pots (18 cm top diameter) filled with a mixture of autoclaved sandy loam soil (sand 70%, silt 22% and clay 8%, pH 7.5) and compost (4:1). For each cultivar two seeds/pot were sown in 8 pots and after 4 d at the two-leaf stage of seedling, thinning was done to maintain one seedling per pot and all the pots except 4 pots of each cultivar were inoculated with 2000 J2 of *M. incognita* into filled with soil 1 cm holes around the base of the plant. There were 4 replicates for each cultivar including control. Uninoculated pot for each cultivar served as controls for that particular cultivar. The pots were watered daily.

Recording of data

Forty-five days after inoculation, the plants were uprooted carefully and root gall indices were determined on a 0~4 scale, where 0=no infection or root galling, 1=slight infection (1%~25%), 2=moderate infection (26%~50%), 3=severe infection (51%~75%) and 4=very severe infection (76%~100%) (Taylor and Sasser, 1978). Fresh weights of root and shoot of each cultivar were obtained just after their uprooting, and dry weights were measured after their drying in an oven at 60 °C for 24 h. Final nematode populations in the entire soil volume were extracted by Cobb's sieving and decanting technique along with Baremann funnel and in roots by macerating 5 g root tissues in a Warring blender (Southey, 1986) and counted by the procedure suggested by Doncaster (1962).

Statistical analysis

Data were subjected to analysis of variance (Cochran and Cox, 1957) and significant difference among cultivars was tested by least significant difference test (LSD) at probability levels of 5% ($LSD_{0.05}$) and 1% ($LSD_{0.01}$). Regression analysis was used to determine whether there was a relationship between root-knot index and total plant fresh weight and dry weight separately.

RESULTS

Among the 23 tested cultivars, no cultivar was highly resistant against *M. incognita*, although, 3 cultivars (HFP-990713, Pant P-25 and HFP-0129) were fairly resistant, cultivars LFP-305, HFP-8909, HUP-31, HFP-4, HFP-0128, Pant P-40, LFP-363, Pant P-31 and HFP-0118 were moderately resistant, cultivar Pant P-2005, Pant P-42 and NDP-2 were tolerant, but the level of tolerance varied among the cultivars. The rest of the cultivars showed moderate susceptibility except cultivar Ambika, where considerable susceptibility towards *M. incognita* was observed. All selections showed great variation in their response to *M. incognita* from resistant to susceptible with 0.25 to 3.25 root-knot index. All field pea cultivars screened for resistance against root-knot nematode favored nematode development in terms of gall index/root system and population densities in soil and root. Lowest root-knot index (0.25) was observed in cultivars Pant P-2005 and HFP-990713, and highest (3.25) in cultivar Ambika. Similarly, lowest final nematode population (668) in soil and root was found in cultivar HFP-990713 whereas highest (10912) was found in cultivar Ambika. Significant difference ($P \leq 0.05$) was also observed in the final nematode population among all the cultivars tested.

Results (Table 1) showed that reproduction of *M. incognita* is favored on susceptible and tolerant cultivars and reduced on resistant ones. According to the rating used for host response, nematode reproduction factor (Rf) was lowest (0.32) in cultivar HFP-990713 followed by Pant P-25 (0.42) as compared to rest of the cultivars.

Variable effects of nematode population on plant growth parameters were observed in all field pea cultivars (Table 2). Reduction in fresh and dry weights of shoot and root from their respective controls confirmed the difference between resistant, tolerant and susceptible cultivars. Maximum reduction in root and shoot fresh and dry weights i.e. 61.8%, 59.3%, 62% and 59.9% respectively were observed for cultivar Ambika, whereas minimum i.e. 2.2%, 2.4%, 2.6% and 2.9%, respectively, in the above parameters was observed for cultivar Pant P-2005. Regression studies showed strong negative relationship between root-knot index and the total plant fresh weight (r=0.9699) as shown in Fig.1, as well as total plant dry weight (r=0.9635) shown in Fig.2.

Table 1 Effect of different *Pisum sativum* cultivars on root-knot development and reproduction of *Meloidogyne incognita*

Selections	Final nematode population (*Pf*)			Reproduction factor (*Rf*)	Root-knot index (*RKI*)*
	Total root*	1 kg soil*	Total		
Pant P-2005	1596	3200	4796	2.40	0.25
HFP-990713	268	400	668	0.32	0.25
Pant P-25	444	400	844	0.42	0.75
NDP-2	654	2000	2654	1.33	1.00
HFP-0129	2472	6000	8472	4.24	1.00
Pant P-42	3120	8000	11120	5.56	1.00
LFP-305	606	2000	2606	1.30	1.25
HFP-8909	712	2400	3112	1.56	1.50
HFP-4	900	2400	3300	1.65	1.50
HUP-31	1164	2400	3564	1.78	1.50
HFP-0128	840	2800	3640	1.82	1.75
Pant P-31	1188	3200	4388	2.19	2.00
Pant P-40	1020	2800	3820	1.91	2.00
LFP-363	1246	3200	4446	2.22	2.00
HFP-0118	900	2800	3700	1.85	2.00
HFP-0110	1056	3600	4656	2.33	2.25
HUDP-28	1072	3600	4672	2.34	2.25
HUDP-15	828	3600	4428	2.21	2.25
HUDP-27	1420	3600	5020	2.51	2.50
HUP-30	1950	3200	5150	2.58	2.75
HUP-2	2200	3600	5800	2.90	3.00
HUDP-26	2268	4000	6268	3.13	3.00
Ambika	4512	6400	10912	5.46	3.25
$LSD_{0.05}$	76.5	216.6			0.60
$LSD_{0.01}$	102.3	289.5			0.80

*Each value is an average of four replicates

Table 2 Effect of *Meloidogyne incognita* (2000 J2/kg soil) on different growth parameters of different cultivars of *Pisum sativum**

Selections	Fresh weight (g)				Dry weight (g)			
	Root		Shoot		Root		Shoot	
	UI	I**	UI	I	UI	I	UI	I
Pant P-2005	13.6	13.3 (2.2)	32.7	31.9 (2.4)	6.99	6.81 (2.6)	15.26	14.82 (2.9)
HFP-990713	13.9	13.4 (3.6)	30.5	29.4 (3.6)	6.87	6.58 (4.2)	13.38	12.82 (4.1)
Pant P-25	11.8	11.1 (5.9)	35.2	32.7 (7.1)	6.26	5.89 (5.9)	15.24	14.42 (5.4)
NDP-2	12.1	10.9 (9.9)	33.5	30.4 (9.3)	6.16	5.58 (9.4)	14.87	13.35 (10.2)
HFP-0129	11.3	10.3 (8.8)	33.7	30.6 (9.2)	6.49	5.87 (9.6)	14.11	12.80 (9.3)
Pant P-42	11.6	10.4 (10.3)	33.6	29.9 (11.0)	5.72	5.12 (10.5)	14.69	13.12 (10.7)
LFP-305	11.7	10.1 (13.7)	34.2	29.4 (14.0)	6.11	5.19 (15.1)	15.66	13.67 (12.7)
HFP-8909	10.6	8.9 (16.0)	32.8	27.2 (17.1)	5.71	4.76 (16.6)	15.55	12.78 (17.8)
HFP-4	10.9	9.0 (17.4)	32.2	26.9 (16.5)	5.28	4.31 (18.4)	15.56	12.74 (18.1)
HUP-31	11.7	9.7 (17.1)	31.3	25.7 (17.9)	5.26	4.38 (16.8)	14.85	12.10 (18.5)
HFP-0128	13.3	10.5 (21.1)	34.8	26.9 (22.7)	6.37	4.91 (22.9)	14.91	12.06 (19.1)
Pant P-31	13.6	9.9 (27.2)	34.2	24.5 (28.4)	6.47	4.85 (25.0)	15.80	11.33 (28.3)
Pant P-40	13.9	10.2 (26.6)	31.8	23.7 (25.5)	6.70	4.88 (27.2)	15.05	11.26 (25.2)
LFP-363	12.1	8.9 (26.4)	31.1	23.0 (26.0)	6.22	4.53 (27.2)	15.89	11.54 (27.4)
HFP-0118	12.1	9.0 (25.6)	31.3	22.8 (27.2)	5.83	4.42 (24.1)	15.27	11.57 (24.2)
HFP-0110	10.3	6.6 (35.9)	33.1	21.1 (36.3)	6.52	4.17 (36.1)	14.80	9.40 (36.5)
HUDP-28	10.4	6.7 (35.6)	30.7	20.0 (34.9)	5.54	3.47 (37.3)	15.94	9.99 (37.3)
HUDP-15	10.6	6.9 (34.9)	30.3	19.6 (35.3)	5.15	3.40 (33.9)	15.05	9.66 (35.8)
HUDP-27	11.8	7.1 (39.8)	33.2	20.5 (38.3)	5.73	3.43 (40.1)	15.21	9.00 (40.8)
HUP-30	12.0	6.5 (45.8)	33.1	17.5 (47.1)	6.14	3.30 (46.3)	16.13	8.73 (45.9)
HUP-2	11.1	5.5 (50.5)	34.1	16.3 (52.2)	5.49	2.71 (50.6)	16.01	8.28 (48.3)
HUDP-26	12.9	6.3 (51.2)	31.8	15.3 (51.9)	6.08	2.96 (51.3)	14.97	7.38 (50.7)
Ambika	12.3	4.7 (61.8)	35.9	14.6 (59.3)	6.27	2.38 (62.0)	15.63	6.27 (59.9)
$LSD_{0.05}$	0.89	1.07	4.04	1.80	0.39	1.14	2.29	0.88
$LSD_{0.01}$	1.20	1.44	5.44	2.42	0.80	1.53	3.08	1.19

UI: Uninoculated; I: Inoculated; *Each value is an average of four replicates; **Values in parenthesis represent percent reduction over uninoculated control

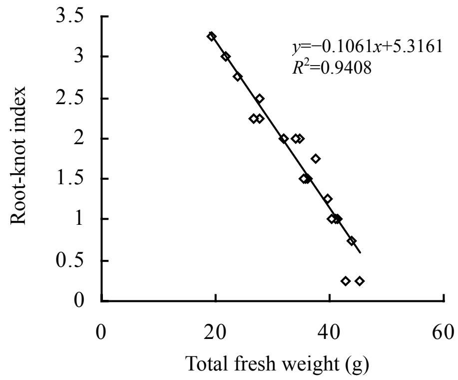

Fig.1 Relationship between root-knot index and total fresh weight of plants of different *Pisum sativum* cultivars

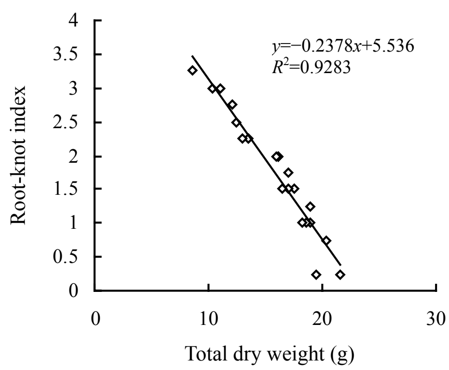

Fig.2 Relationship between root-knot index and total dry weight of plants of different *Pisum sativum* cultivars

DISCUSSION

Use of resistant cultivars for the management of nematode population is expected to be a vital management component in the future. In the present study, plant response to *M. incognita* was measured by amount of galling and estimating soil and root densities. There was considerable variation in response against *M. incognita* among the different cultivars of field pea screened. No cultivar was found highly resistant against *M. incognita*, although resistance (*RKI*=0.25−1.00) and moderate resistance (*RKI*=1.25−2.00) reaction was shown by some cultivars. Determination of gall index is not as laborious as determination of reproduction. Thus, breeding programs would be best served to select resistant genotypes based on root-knot index in preliminary evaluations, followed by selection based on nematode reproduction in advanced evaluations (Hussey and Janssen, 2004). It was also observed that there was a wide range of tolerance expressed among the moderately resistant and resistant cultivars. Such variability in tolerance might be influenced by host plant genetics and other environmental factors that can affect plant growth. Tolerance levels of cultivars with similar gall ratings and resistance levels could differ if galling on one cultivar was somehow more disruptive of root function (Davis and May, 2003), as we have found in the case of cultivar NDP-2 and Pant P-42, where root-knot index in both cultivars was 1.00. Results showed that the nematode population used for the disease induction suppressed the fresh and dry weights of root and shoots of all tested cultivars. Presence of nematode resistance genes makes the plant root less attractive for attacking nematodes. There may be possibility of interception of signal transduction in recognition event. Regression analysis in our study indicated that as the level of resistance increases, the level of nematode tolerance increases. This relationship between resistance and tolerance may be considered analogous to a damage function relating nematode population density and damage. Resistance and susceptibility to plant parasitic nematodes reflect the effect of the plant on the nematode's ability to reproduce (Cook and Evans, 1987) as our results indicated that on cultivars, HFP-990713, Pant P-25 and NDP-2, reproductions of nematodes were lowered as compared to other cultivars. Whereas tolerance and intolerance reflect the degree of damage inflicted by the nematode on the plant (Cook and Evans, 1987), as we have found in the case of selections HFP-0129 and Pant P-42, on which high nematode population caused less damage than that on other cultivars, wherein lower population caused more damage.

Nematode resistance should contribute to nematode management, especially when combined with nematode tolerance as we have observed in cultivar HFP-0129 and Pant P-42. Barnes *et al*.(1990), released two alfalfa germplasms that exhibited superior performance in *Pratylenchus penetrance* infested fields. The germplasm had 20%~30% fewer nematodes/g of fresh root (moderately resistant) and exhibited tolerance. Resistant and moderately resistant cultivars may be most valuable if they reduce nematode reproduction enough to affect the residual nematode population density in a field.

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