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Incidence and Reproduction of *Meloidogyne incognita* on Vegetable Crop Genotypes

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Abstract.- In 2006-2008, a survey was conducted in 16 major vegetable production areas of the Punjab with the purpose of determining the incidence and distribution of *Meloidogyne incognita* and its reproduction on vegetable crop genotypes in nematode infested fields and in the green house. Two root knot nematode species. *M. incognita* and *M. javanica,* were identified from 260 samples. *Meloidogyne incognita* was the predominantly found species and was detected in 13.6% of all the fields surveyed. In the field, the average nematode incidence was 41.5% and ranged from 5.4% in fields planted with mustard to 94.4% in fields planted with okra. The root gall severity averaged 5.5 on scale of 0 to 9. Seventeen vegetable genotypes were evaluated for resistance to gall formation and reproduction by *M. incognita*. Resistance was identified only in cauliflower, mustard, and radish. The most susceptible genotypes were bitter gourd, carrot, cucumber, eggplant, lettuce, okra, pea, pumpkin, sponge gourd and watermelon meanwhile; three plant species cabbage, chilies, and coriander provided an intermediate host response. Some plant genotypes appeared to be hypersensitive as they exhibited heavy root galling but suppressed nematode reproduction. These included members of family Cucurbitaceae.

Keywords: Host suitability, *Meloidogyne incognita, M. arenaria,* reproduction factor.

INTRODUCTION

Root-knot nematodes, *Meloidogyne* species, are parasitic on a wide variety of plant hosts and are especially common in warmer sandy soils of the Punjab. The nematode infection induces extensive galling and root damage. Vegetable crops usually are among the most susceptible and worst affected by these nematodes (Sharma *et al*., 2006; Anwar *et al*., 2007; Singh and Khurma, 2007). Infection of roots by nematodes alter uptake of water and nutrients and interferes with the translocation of minerals and photosynthates (Williamson and Hussey, 1996). Such alterations change the shoot: root ratio (Anwar and Van Gundy, 1989) and expose the plants to other pathogens. For example, nematode root infection increases the incidence and severity of *Fusarium* wilt diseases on a variety of crops (Anwar and Khan, 1973; Martin *et al*., 1994), which can negatively influence yield (Orr and Robison, 1984). Vegetable yield reductions have reached as high as 30% for susceptible genotypes in the presence of plant parasitic nematodes in some production areas (Anwar *et al*., 2009a). Root systems may be deformed, and underground organs such as potato tubers and carrot taproots may be damaged and become unmarketable (Roberts, 1987; Sikora and Fernandez, 1990). In 2005, three fields in Faisalabad, Punjab, were observed exhibiting chlorotic and necrotic stunted plants with heavily galled root systems. Losses in sponge gourd and squash in those fields exceeded 60%.

Meloidogyne incognita is a damaging pathogen of vegetables and has been predominantly found infecting vegetable crops in warmer climates. *Meloidogyne incognita* has been found infecting a wide range of crops in Baochistan (Khan *et al*., 2005), North West Frontier Province (Gul and Saeed, 1990), Punjab (Anwar *et al*., 2007) and Sind (Sattar *et al*., 1987).

The suitability of a host for plant parasitic nematodes is expressed as the ability of the nematode to multiply on the plant. The host status may be determined by their reproduction factor (Pf/Pi = final population of nematodes per initial population) and nematodes per gram of root have been widely used in nematological studies to define resistance and susceptibility of plants to nematodes, which is frequently used as the indicator of the nematode-host relationship (Bélair and Benoit, 1996; McKenry and Anwar, 2006). Genotypes can

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also be evaluated for root-knot nematode resistance based on the degree of root galling, egg mass number or total number of eggs collected per root system (Hussey and Boerma, 1981).

Increased information on frequency and distribution of root-knot nematodes in vegetable production areas and their reproduction on vegetable crop genotypes is important for sustainable production of vegetables because the use of resistant cultivars is considered one of the best and environmentally safe alternatives among the non-chemical methods. The objectives of this study were to: 1. determine the incidence and distribution of *M. incognita* in the vegetable production area of the Punjab and 2. reproduction on commercially used vegetable crop genotypes. This study will increase our understanding of host suitability among various vegetable genotypes common to Pakistan.

MATERIALS AND METHODS

Nematode survey

Seventeen vegetable crop genotypes planted in 16 major vegetable production areas in the Punjab were evaluated during 2006-2008 to determine the presence of root-knot nematodes, *Meloidogyne* spp. (Table I). The crops evaluated were bitter gourd, cabbage, carrot, chillies, coriander, cucumber, lettuce, mustard, okra, pea, pumpkin, radish, sponge gourd, tomato and watermelon. In the top 15 to 20-cm, soils averaged 65% to 85% sand, 5.5% to 20.25% silt, 10% to 21.5% clay, 0.5% to 2.75% organic matter.

A total of 260 randomly selected fields from 16 different localities was surveyed for the presence of root- knot nematodes. At each location, root and soil samples consisting of a composite of 10 soil cores were collected from the rhizosphere zone of ten stunted and wilted plants from each field using an Oakfield tube (2.5-cm diam x 30-cm deep). The samples were labeled with the host plant, locality, date and grower's name, immediately put in a cooler for transport and eventually stored at 4°C until processed.

Roots were separated from soil, carefully washed under tap water to remove adhering soil particles and towel dried before weighing. Nematodes were extracted from a fresh root composite sub-sample of 20g by placing in a mist-chamber for 5 days (McKenry and Roberts, 1985).

Table I.- Vegetable crops and localities sampled to assess the root knot nematode infestation.

Vegetable genotypes	Localities sampled*
Tomato (*Lycopersicon esculentum*)	Attock, Chakwal, D.G. Khan, Faisalabad, Gujranwala, Khanewal, Kushab, Lahore, Layyaha, Multan, Okra, Rahimyar Khan, Sargodha, Sheikhpura, Sialkot, Taunsa-sahrif
Okra *(Abelmoschus esculentus)*	Chakwal , D.G. Khan, Faisalabad, Kushab, Layyaha, Multan, Rahimyar Khan, Sargodha, Sheikhpura, Sialkot, Taunsa-sahrif
Chillies (*Capsicum annuum*)	Attock, Chakwal, Faisalabad, Gujranwala, Khanewal, Multan, Okra, Rahimyar Khan, Taunsa-sahrif
Egg plant (*Solanum melongena*)	D.G. Khan, Faisalabad, Gujranwala, Kushab, Okra, Rahimyar Khan, Sargodha, Sheikhpura, Taunsa-sahrif
Pumpkin (*Cucurbita argyrosperma*)	Attock, Faisalabad, Lahore, Rahimyar Khan, Sargodha, Sialkot
Sponge gourd (*Luffa cylindrica*)	Faisalabad, Khanewal, Lahore, Layyaha,
Watermelon (*Citrullus lanatus*)	Faisalabad , Khanewal, Taunsa-sahrif
Cauliflower (*Brassica oleracea*)	Faisalabad , Gujranwala, Lahore, Sargodha, Sialkot
Carrot (*Daucus carota*)	Faisalabad , D.G. Khan, Gujranwala, Kushab, Okra, Rahimyar Khan, Sargodha
Cabbage (*Brassica campestris*)	Faisalabad , Kushab
Mustard (*Raphanus sativus*)	Faisalabad , Chakwal, Kushab, Rahimyar Khan
Radish (*Raphanus sativus*)	Faisalabad , Kushab
Cucumber (*Cucumis sativus*)	Faisalabad , Sheikhpura
Bitter gourd (*Momordica charantia*	Faisalabad , Sheikhpura
Pea (*Pisum sativum*)	Attock, Faisalabad , D.G. Khan
Coriander (*Coriandrum sativum*)	Faisalabad
Lettuce (*Lactuca sativa*)	Faisalabad

The population of *Meloidogyne* juveniles was quantified under a dissecting microscope at 40X magnification. Sub-samples were used to propagate the nematode populations in pots in a greenhouse to study the host suitability of the vegetable crops to *M. inocgnita*.

Identification of root knot nematodes

Fifteen-days-old seedlings of tomato (*Lycopersicon esculentum* Mill cv. Money Maker) were transplanted into the nematode-infested soil in 15-cm clay pots. Forty five days after inoculation, a single egg mass was hand picked from the galled roots and used to inoculate 15-day-old tomato plants of cv. Money Maker and allowed to grow for 60 days in the greenhouse at an average temperature of 30 ± 3°C. Root-knot nematodes were identified using perineal patterns of adult females as well as the morphology of second stage juveniles (Hartman and Sasser, 1985; Jepson, 1987).

Nematode inoculum

Meloidogyne incognita, originally isolated from single egg masses, was increased on tomato cv. Money Maker in a green house. Eggs were collected from roots of tomato by placing in 800 ml sealed Manson glass jar with 1% NaOCl (Hussey and Barker, 1973), and shaken for 4 min at 200 cycles/min on a mechanical shaker (Eberbach Corporation, Ann Arbor, MI). This treatment was followed by a thorough rinse in tap water and egg was counted at 40X magnification. Suspensions of eggs were stirred in tap water and counts adjusted to enable the desired inoculum density to be added per pot.

Evaluation of crop genotypes

Fifteen days old seedlings and young plants of vegetable crop genotypes were inoculated with 5000 eggs and hatched second stage juveniles (J2) at transplanting. The nematode suspension was poured into four holes about 3-cm deep around the base of each plant. The holes were then filled with soil and a little water was added to the pots. There were three replicates of each genotype. Pots were arranged in a completely randomized design in a greenhouse with temperature ranging from 30±3°C and a 14 hr photoperiod. Plants were fertilized every two weeks with Hoagland's solution (Hoagland and Arnon, 1950).

Experiments were terminated 60 days after inoculation. Roots were washed free from soil, and root systems of the plants were stained with Phloxine B (Holbrook *et al*., 1983) and assessed for the presence of egg masses. The root systems were rated for galling and egg mass indices on a 0 to 5 scale (Quesenberry *et al*., 1989), where 0 = no galls or egg masses, 1 = 1 or 2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 > 100 galls or egg masses per root system. The root galling severity was assessed on 0 to 9; 1 = no galling 2 = <5% roots galled, 3 = 6-10%, 4 = 11-18%, 5 = 19-25%, 6 = 26-50%, 7 = 51-65% 8 = 66-75%, and 9 = 76-100% roots galled (Schoonhoven and Voysest, 1989). Eggs were extracted from each root system and counted to determine final population density for each plant. Nematodes from roots were extracted as above. Nematodes per gram of root were calculated to determine the reproductive ability of nematode on each vegetable genotype. Host suitability was categorized as good [susceptible] when Pf/Pi > 5.0, fair [moderately resistant] if 5.0 ≥ Pf/Pi > 1, poor if 1 > Pf/Pi > 0, and nonhost when Pf/Pi = 0 (Zhang and Schmitt, 1994)

RESULTS AND DISCUSSION

Identification of root-knot nematodes

Two species of root-knot nematode, *M. incognita* and *M. javanica,* were identified from 260 samples at 16 localities. *Meloidogyne incognita* was found in 90% of vegetable fields with an average gall and egg mass indices of 3.5 and 3.25 on 0 to 5 scale, respectively. Most of the samples were mixed with both species. *Meloidogyne javanica* was detected only in 7% of the samples. *Meloidogyne incognita* is to be considered as the most damaging pathogen of vegetable crops in Pakistan (Anwar *et al*., 1992a) and worldwide (Sikora and Fernandez 1990).

Incidence and distribution of M. incognita

Meloidogyne incognita was detected in 16 localities sampled (Table II). Percentage nematode infested fields found in the localities ranged from 7% in Multan and to a high of 27% in Faisalabad.

The percentage of infested fields in Faisalabd, Layyaha, and Sialkot was 27%, 20%, and 20%, respectively. Fields exhibiting chlororotic foliar symptoms and necrotic stunted plants were observed in these three localities. Across these 16 localities, an average of 13.6% sampled was infested with *M. incognita*.

Incidence of M. incognita *on vegetable crops in the field*

The incidence and root galling severity varied among the plant genotypes and the location of sampling. The average nematode incidence among seventeen vegetable genotypes was found to be 41.5%. The percentage incidence from the different localities ranged from 5.4% in fields planted with mustard up to 94.4% in fields planted with okra (Table III). A total of 17 commonly grown crop genotypes planted at various locations in the Punjab were found infected by root-knot nematodes (Tables I, III).

Although only 17 crops were included, this survey indicates the existence of root-knot nematode populations in the agricultural production area of the Punjab. A more detailed survey is needed to reveal additional crops and weeds that serve as nematode hosts (Anwar *et al*., 1992b). The reasons for the fairly widespread distribution of root-knot nematode might be their extensive host range, including weeds (Anwar *et al*., 2009c; Sikora and Fernandez 1990), the lack of awareness among growers about nematodes, the unintentional spread through sharing of seedlings and farm implements and tropical climatic conditions favoring the build-up of root-knot nematode populations.

Root galling severity on scale of 0 to 9 indicated infestation on roots of 17 vegetable genotypes by root-knot nematodes that ranged from severe (8.5) on roots of egg plant to light (1.5) on roots of cauliflower with an average of 5.5 across all the crop genotypes (Table III). Our results concur with other reports on nematodes of vegetables (Fassuliotis, 1970; Potter and Olthof, 1993). Root galling severity is a measure of the size of the nematode population. High severity impacts foliar growth by inducing various physiological alterations in plant vital functions. The end result of poor foliage growth is ultimately reduced yield (Melakeberhan and Webster, 1993; Anwar, 1995).

Table II. Localities sampled for nematodes, year sampled, samples collected and infested fields with *Meloidogyne incognita*

Localities	Year	Samples collected	Infested fields	
			No. of fields	Percentage
Attock	2006	19	3	10.50
Chakwal	2006	18	2	11.0
D. G. Khan	2007	8	1	12.5
Faisalabad	2006	45	15	33.0
Gujranwala	2006	21	2	18.0
Khanewal	2008	11	1	9.0
Khushab	2006	5	0	0.0
Lahore	2008	20	3	15.0
Layyaha	2006	20	4	20.0
Multan	2006	15	1	7.00
Okara	2007	16	2	12.50
Rahimyar Khan	2007	10	1	10.0
Sargodha	2006	15	2	13.0
Sheikhupura	2006	18	2	11.0
Sialkot	2008	5	1	20.0
Taunsa-sahrif	2007	14	2	14.3
Total	2006-2008	260	42	Average = 13.6

Vegetable crop evaluation against M. incognita *in a green house*

Meloidogyne incognita has a broad host range. It reproduced on 14 of 17 genotypes (82%) from 8 botanical families with a reproductive factor [RF =Pi/ Pf] >1 (Table IV). Six genotypes (35.3%) were categorized as good hosts (susceptible) and included carrot, lettuce, pea, okra, egg plant and tomato. The average J2 population per g of root on these hosts arranged from 201 in lettuce to 450 in tomato. Tomato was the best host with highest RF of 125.5 (P= 0.05). Theses findings agree with that reported by others for vegetables (Mani and Al-Hinai, 1996; Sharma *et al*., 2006; Brito *et al*., 2007).

Those listed as moderate hosts (RF < 5.0 but > 1) accounted for 47% of the genotypes tested and included coriander, cabbage, bitter gourd, cucumber, pumpkin, sponge gourd, watermelon and Chile. The greatest J2 population per g root was on watermelon.

Three genotypes were categorized as poor hosts. Their RF ranged from 0.9 in radish to 0.7 in

Table III.- Incidence and root galling severity of *Meloidogyne incognita* on seventeen vegetable genotypes grown in the fields.

Family	Common name	Scientific name	Incidence %	Root gall severity [1-9]*
Apiaceae	Carrot	*Daucus carota*	60.0	7.5
	Coriander	*Coriandrum sativum*	12.3	2.5
Asteraceae	Lettuce	*Lactuca sativa*	20.4	7.5
Brassicaceae	Cauliflower	*Brassica oleracea*	13.6	1.5
	Radish	*Raphanus sativus*	6.7	2.0
Cruciferae	Cabbage	*Brassica campestris*	7.8	2.3
	Mustard	*Sinapis alba*	5.4	1.5
Cucurbitaceae	Bitter gourd	*Momordica charantia*	14.0	8.5
	Cucumber	*Cucumis sativus*	35.0	7.4
	Pumpkin	*Cucurbita argyrosperma*	57.4	8.5
	Sponge gourd	*Luffa cylindrica*	75.2	8.0
	Watermelon	*Citrullus lanatus*	80.6	6.7
Fabaceae	Pea	*Pisum sativum*	10.7	6.0
Malvaceae	Okra	*Abelmoschus esculentus*	94.4	7.0
Solanaceae	Chilies	*Capsicum annuum*	23.0	3.0
	Egg plant	*Solannum melongena*	75.4	7.5
	Tomato	*Lycopersicon esculentum*	85.8	8.0
		Mean	41.5	5.5

*Rooting galling severity scale where: 1, no galling; 2, <5% roots galled; 3, 6-10%; 4, 11-18%; 5, 19-25%; 6, 26-50%; 7, 51-65%; 8, 66-75%; and 9, 76-100% roots galled (Schoonhoven and Voysest, 1989).

Table IV.- Gall and egg mass indices, J2 population, reproduction factor (RF) of *Meloidogyne incognita* on seventeen vegetable genotypes 60-days after inoculation with an initial population density (Pi) of 5000 eggs per plant in the greenhouse.

Family	Common name	Scientific name	Indices*		RF** [Pf/ Pi]	J2 / g root	Host status
			Galls	Egg masses			
Apiaceae	Carrot	*Daucus carota*	5.0	4	25.5	320	S
	Coriander	*Coriandrum sativum*	3.5	3	3.5	87	MR
Asteraceae	Lettuce	*Lactuca sativa*	5.0	4	45.0	201	S
Brassicaceae	Cauliflower	*Brassica oleracea*	1.0	1	0.8	3	R
	Radish	*Raphanus sativus*	1.0	1	0.9	3	R
Cruciferae	Cabbage	*Brassica campestris*	3.0	1	1.6	3	MR
	Mustard	*Sinapis alba*	1.0	1	0.7	3	R
Cucurbitaceae	Bitter gourd	*Momordica charantia*	5.0	4	2.5	40	S
	Cucumber	*Cucumis sativus*	5.0	4	3.5	45	S
	Pumpkin	*Cucurbita argyrosperma*	5.0	4	1.5	27	S
	Sponge gourd	*Luffa cylindrica*	5.0	4	4.5	16	S
	Watermelon	*Citrullus lanatus*	5.0	4	2.5	112	S
Fabaceae	Pea	*Pisum sativum*	5.0	4	34.5	302	S
Malvaceae	Okra	*Abelmoschus esculentus*	5.0	4	60.0	276	S
Solanaceae,	Chilies	*Capsicum annuum*	2.5	2	2.5	76	MR
	Egg plant	*Solanum melongena*	5.0	4	75.5	350	S
	Tomato	*Lycopersicon esculentum*	5.0	4	125.5	450	S

*, Data are mean of three replications; **RF, Reproduction factor whereas Pf is final nematode population density divided by initial nematode population density.

mustard. Their lower gall and egg mass indices per root system suggests that these three genotypes are resistant.

The five plant genotypes from the family Cucurbitaceae, bitter gourd, cucumber, pumpkin, sponge gourd and watermelon, tend to be excellent

hosts in terms of high gall and egg mass indices (Anwar *et al*., 2007; Zhang and Schmitt, 1994). However, due to their low RF and J2 per g of root they were ranked as fair hosts to *M. incognita*. This suggests that J2 were able to penetrate roots, develop to egg-laying adult females and induce root galling but their egg hatching might be reduced by some root factors. Root exudates from some plants exert a suppressive effect on root-knot nematode reproduction (Vicente and Acosta, 1987). The factors responsible for reduced reproduction *M. incognita* on these plant genotypes should be elucidated.

The findings of this study have clearly demonstrated that *M. incognita* is widely distributed across the state of the Punjab and involves a wide host range. *Meloidogyne incognita* was found associated with 17 plant species belonging to eight families (Apiaceae, Asteraceae, Brassicaceae, Cruciferae, Cucurbitaceae, Fabaceae, Malvaceae, and Solanaceae). Our results agree with that reported from Sultanate of Oman parasitizing bitter gourd, egg plant, okra, pepper, and tomato (Mani and Al-Hinai, 1996) and cabbage, carrot, cucumber, pea, pumpkin, sponge gourd, water melon, and melon (Johnson, 1998).

Our results also provide important information for scientists and awareness to growers about the occurrence of root-knot nematode infecting crops. This investigation has identified the potential of rotation crops like cauliflower, radish and mustard, which could be used to manage the root-knot nematodes by planting on fields previously planted with susceptible host crops.

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