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Screening of Tomato Genotypes for Root Knot Nematode (*Meloidogyne incognita* Kofoid and White Chitwood)

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A B S T R A C T

A study was conducted to evaluate the reaction of tomato genotypes to root knot nematode (*Meloidogyne incognita* race - 3) and the nematode reproduction were studied in the nematode infested pot culture experiment. Forty tomato genotypes were subjected for screening. At 65 days after nematode inoculation, whole plants were uprooted, washed and ranked for root galling and egg mass indices on a 1 to 5 scales. The plant growth responses *viz*., root length, root dry weight and nematode reproduction in term of number of galls per gram of root system, gall index, egg masses, eggs per egg mass, and second stage juveniles per 200-cm^3 of soil were recorded. The field experiment revealed that *M. incognita* was able to induce root galling and reproduced on all the forty tomato genotypes screened. All the tomato genotypes show varying degree of response. Out of forty genotypes of tomato used in this experiment Hisar Lalit, HN 2, PNR 7, IIHR 2614 and IIHR 2868 were found to be resistant to root knot nematode and these cultivars can be used as a source of resistance. However, the tomato varieties usually cultivated in Tamil Nadu are highly susceptible to root-knot nematode and thus provide substrate for buildup of population of root knot nematode in tomato field. These varieties should be replaced in order to reduce the population of root knot nematode. The use of resistant varieties to manage the population of nematode is very cost effective method to control the plant parasitic nematodes.

Keywords

Tomato, Nematode, Meloidogyne incognita, Resistance.

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Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most popular vegetable crops worldwide, owing to its high nutritive value and diversified use. Plant parasitic nematodes are important pests of tomato and cause huge economic losses (Bird and Kaloshian, 2003). Root knot nematodes (*Meloidogyne* spp.) are a concern to both smallholders and commercial producers involved in intensive tomato cultivation. Damage to plants is influenced by root penetration, development, reproduction potential and inoculums density of *M. incognita* in adjacent soil (Shahab and Sharma, 2011). High densities of the nematodes at planting induce loss of foliage and root growth and severe root galling (Barker, 1998).

Meloidogyne incognita is a major pest of tomato and they cause damage by feeding and inducing large galls or "knots" throughout the root system of infected plants, which can interfere with the uptake of water and nutrients and thereby translocation of photosynthates is drastically affected (Anwar *et al.,* 2010). It also alters the host physiology and on severe infestations can kill the tomato plant outright (Kamran *et al.,* 2010). The degree of root galling generally depends on the magnitude of *Meloidogyne* population density, host plant species and cultivar. Severe nematode infections result in decreased yield of tomato and the quality of the marketable products is reduced and cause tissue breakdown, deformation or discoloration. *M. incognita* has been found in most of the tomato growing areas of the world, which impacts tomato yields (Anwar and McKenry, 2010). It can cause up to 5 per cent yield losses globally (Cetintas and Yarba, 2010).

The use of resistant varieties is a promising method of controlling plant parasitic nematodes and the resistance is often managed by one or more genes in tomato cultivars (Amati *et al.,* 1985). It has been found that root knot nematodes may enter susceptible and resistant tomato varieties in about equal numbers. Hence breaking of resistance in tomato cultivars to *M. incognita* may occur naturally or by selection of tomato plants with one or more resistant genes (Khan and Nirupma, 2000).

Several management strategies including use of chemicals and crop rotation have been used extensively over the years to minimize the losses caused by nematodes, but these strategies have their limitations (Sharon *et al.*, 2001). Continuing environmental problems associated with the use of nematicides and unreliable results from crop rotation systems have resulted in a sense of urgency regarding the search for alternative nematode management strategies (Kerry, 1990). The primary objective of the current research was to evaluate the available and resistant root knot nematode tomato germplasm against *M. incognita* by artificial inoculation method and screening of resistant genotypes to root knot nematode for further studies.

Materials and Methods

Experimental procedure

The seeds of forty tomato breeding genotypes were obtained from various State Agricultural Universities. Pot culture experiment was conducted under glasshouse condition at the Department of Vegetable crops, HC and RI, TNAU, Coimbatore during 2015. The seedlings were raised in protrays and twenty five days old healthy seedlings were transplanted in earthen pots containing two and half kilogram of sterilized pot mixture (Red soil: Sand: FYM in 2:2:1 ratio) for artificial inoculation of root knot nematode. The experiment was laid out in a Completely Randomized Design with three replications.

Root knot nematode infected tomato plants were collected from the Department of Nematology, TNAU, Coimbatore. The identity of species *M. incognita* race 3 was confirmed by using taxonomic keys and host differentials. Highly susceptible tomato cultivar PKM 1 was used for developing pure culture of root knot nematode. Plants of PKM 1 tomato were raised in the pots filled with steam sterilized loamy soil mixed with fine river sand. The potted plants were inoculated with one J_2 stage of *M. incognita* @ per gram of soil and maintained as pure culture.

Nematode inoculation

The method of Sasser *et al.,* (1957) was followed for inoculating nematodes. Infected roots from pure culture were cut into small pieces of about 2 cm long and placed in sodium hypochlorite (NaOCl) 0.5 per cent solution. The container was shaken for about 3 minutes to dissolve the gelatinous matrix to separate the eggs from egg mass and incubated for 48 hours under laboratory condition for hatching. The eggs were kept in Petri dishes and frequently aerated with the use of aerator to enable hatching. The concentration of hatched out J_2 was adjusted to a known number by addition of water for inoculation. The nematode inoculum (J_2) was inoculated at 2 cm depth near rhizosphere and covered with sterile sand. Each pot was inoculated with J_2 of *M. incognita* at the rate of two juvenile (J_2) / g of soil on 15 days after planting.

Assessment of nematode population and root knot index

Sixty days after inoculation, seedlings were uprooted carefully with minimum root damage and washed with tap water to remove the adhering soil particles. Plant growth parameters *viz*., root length and dry weight were measured. Dry weight was determined after drying the plants in a hot air oven at 60°c for 72 hours. From the fresh root sample, number of females per gram of root, number of egg mass per gram of root and number of eggs per egg mass were counted under a stereoscopic microscope after staining with acid fuchsin lactophenol.

Assessment of root knot nematode resistance (Root gall indexing)

The degree of resistance was indicated by the root knot index and it was done as per Heald *et al*., (1989) (Table 1).

Results and Discussion

Out of forty genotypes of tomato screened, five genotypes *viz*., Hisar Lalit, HN 2, PNR 7, IIHR 2614 and IIHR 2868 were found to be resistant which recorded a root knot index of 2.0 (Table 1), while the seven genotypes *viz*., IIHR 550-3, IIHR 915, IC 249505, IC 249515, IC 550742, IC 567277 and IC 567307 were found to be moderately resistant. Twenty five genotypes *viz*., IC 249504, IC 249506, IC 249507, IC 249508, IC 249511, IC 249512, IC 249513, IC 249514, IC 549828, IC 549835, IC 567346, CLN 2123A, PKM 1, CO 3, Hisar Arun, Anahu, Punjab Kesari, Punjab Chhuhara, Punjab Ratta, Punjab Upma, Arka Ashish, Arka Alok, Arka Meghali, Arka Vikas and Arka Saurabh were found to be susceptible and three genotypes *viz*., IC 249503, Arka Abha and LE 812 were found to be highly susceptible to root knot nematode which recorded highest number of root gall index, number of females, number of egg masses and number of eggs.

The nematode resistant plants are characterized by failure of the nematodes to produce functional feeding sites in the host after invasion and to develop subsequently as reproducing females, including hypersensitive responses (Williamson and Kumar, 2006). Two types of mechanisms for nematodes resistance in plants have been reported, including pre-infection resistance, where the nematodes cannot enter the plant roots due to the presence of toxic or antagonistic chemicals in root tissue (Bendezu and Starr, 2003), and post-infection resistance in which nematodes are able to penetrate roots but fail to develop (Anwar and McKenry, 2000). Post-infection resistance is often associated with an early hypersensitive reaction (HR), in which rapid localized cell death in root tissue around the nematode prevents the formation of a developed feeding site, leading to resistance. Tomato plants that are resistant show typical HR upon avirulent RKN infection (Williamson, 1999) Boiteux and Charechar (1996) reported that resistant genotypes have gene of resistance in their gene pool which confers resistance to *M.*

incognita. Resistance and susceptibility to *M. incognita* reflect the effect of the plant on the nematode's ability to reproduce (Cook and Evans, 1987). In the genotypes *viz*., Hisar Lalit, HN 2, PNR 7, IIHR 2614 and IIHR 2868 reproduction of nematodes was lower as compared to other genotypes. The compatible reaction of the remaining tomato genotypes to *M. incognita* infection indicated that they lack resistant genes so genotypes were unable to stop the penetration, development, and reproduction. This suggests that we need to transfer resistant genes to tomato genotypes to avoid the infection by nematodes, which is essential for the management of root knot nematodes.

Meloidogyne incognita was able to induce root galling on the roots of all the tomato genotypes but at differential rates, which might be due to differences in genetic make-up among the genotypes (Jacquet *et al.*, 2005). High root gall indices (4 to 5) for all twenty seven tomato genotypes rendered them as good host of *M. incognita*. Root galling indices have been used to assess host status of annual and perennial crops to root-knot nematodes (Zhou *et al.*, 2000). Whereas lowest root gall index (2) were found in the resistant tomato genotypes of Hisar Lalit, HN 2, PNR 7, IIHR 2614 and IIHR 2868. However, root gall index is not a satisfactory indicator of the durability of root-knot nematode resistance (Hussey and Boerma, 1981; Reed and Schneider, 1992; Zhou *et al.*, 2000). Whereas the parameters *viz.*, number of females, number of egg masses and number of eggs per gram of root recorded in tomato genotypes are the better indicators of nematode reproduction than root gall index (Ornat *et al.*, 2001).

Number of females were found to be maximum in the highly susceptible genotypes *viz*., Arka Abha (37.00), IC 249503 (29.66) and LE 812 (27.33) whereas, minimum number of females were observed in the resistant genotypes *viz*., Hisar Lalit (4.00), PNR 7 (7.33), HN 2 (8.33), IIHR 2614 (9.33) and IIHR 2868 (11.33). The population of females per gram of root was significantly increased in highly susceptible and susceptible tomato genotypes and that was decreased in moderately resistant and resistant tomato genotypes.

Maximum number of egg masses per root system was obtained in three highly susceptible genotypes *viz*., Arka Abha (40.66), IC 249503 (36.53) and LE 812 (34.33) whereas minimum number of egg mass per gram of roots were observed in the resistant genotypes *viz*., Hisar Lalit (5.00), PNR 7 (8.00), HN 2 (15.33), IIHR 2614 (18.66) and IIHR 2868 (19.33).

Table.1 Assessment of root knot nematode resistance (Root gall indexing)

Percentage of roots with galls	**Root knot index**	**Reaction**
0	1	Highly resistant (HR)
1-25	2	Resistant (R)
26-50	3	Moderately resistant (MR)
51-75	4	Susceptible (S)
76-100	5	Highly susceptible (HS)

Table.2 Screening of tomato genotypes to root knot nematode (*Meloidogyne incognita*)

Genotypes	Root knot index	No. of females / g root	No. of egg masses / g root	No. of eggs / egg mass	Root length (cm)	Root dry weight (g)
IIHR 550-3	3.0	13.53	22.00	110.00	32.53	4.12
IIHR 915	3.0	18.00	22.66	120.00	31.56	4.00
IIHR 2614	2.0	9.33	18.66	92.33	35.33	4.35
IIHR 2868	2.0	11.33	19.33	101.33	35.20	4.25
IC 249503	5.0	29.66	36.53	285.66	10.35	0.87
IC 249504	4.0	18.33	29.66	199.33	21.90	1.55
IC 249505	3.0	15.33	23.33	126.00	26.50	3.10
IC 249506	4.0	20.00	30.66	208.00	21.60	1.48
IC 249507	4.0	20.66	35.66	271.00	17.83	1.26
IC 249508	4.0	20.00	36.33	278.00	16.60	1.22
IC 249511	4.0	20.21	31.33	210.00	21.40	1.44
IC 249512	4.0	19.66	34.33	236.66	20.43	1.37
IC 249513	4.0	18.00	34.33	237.00	19.60	1.34
IC 249514	4.0	20.00	34.00	235.33	21.00	1.41
IC 249515	3.0	17.86	26.66	137.66	25.00	2.20
IC 549828	4.0	19.00	37.00	280.00	16.00	1.12
IC 549835	4.0	20.33	36.53	279.33	16.30	1.20
IC 550742	3.0	17.34	24.00	130.00	25.93	2.45
IC 567277	3.0	18.00	24.45	131.00	25.65	2.40
IC 567307	3.0	17.66	26.33	138.00	25.50	2.23

IC 567346	3.0	15.33	22.66	120.66	26.9	3.22
HN 2	2.0	8.33	15.33	92.33	36.70	4.36
CLN 2123A	4.0	20.21	26.66	139.00	24.76	2.16
PKM 1	4.0	24.66	27.00	170.00	22.83	2.07
CO 3	4.0	24.66	39.33	280.00	10.40	1.05
Hisar Lalit	2.0	4.00	5.00	64.66	40.50	5.35
Hisar Arun	4.0	23.00	26.66	139.66	24.40	2.14
Anahu	4.0	24.00	27.00	150.00	24.10	2.13
Punjab Kesari	4.0	18.00	38.66	284.00	15.00	1.09
Punjab Chhuhara	4.0	26.66	29.33	185.33	22.43	1.98
PNR 7	2.0	7.33	8.00	90.00	40.33	5.06
Punjab Ratta	4.0	26.22	38.33	210.00	23.90	1.15
Punjab Upma	4.0	21.00	29.33	184.66	22.26	2.00
Arka Ashish	4.0	24.33	27.66	183.00	22.56	2.00
Arka Abha	5.0	37.00	40.66	290.00	10.13	0.21
Arka Alok	4.0	24.00	27.33	166.00	23.16	2.08
Arka Meghali	4.0	23.33	27.33	158.00	23.23	2.10
Arka Vikas	4.0	23.66	27.00	153.33	23.4	2.13
Arka Saurabh	4.0	23.33	28.33	185.33	22.13	1.55
LE 812	5.0	27.33	34.33	282.33	14.6	1.05
Mean	3.67	19.83	28.64	183.41	23.20	2.15
SE(d)	0.61	3.40	4.85	32.00	3.97	0.40
SE	0.09	0.53	0.76	5.10	0.62	0.06
CD(0.05)	1.22	6.74	9.66	63.69	7.91	0.80

Number of eggs per egg mass was significantly increased in highly susceptible and susceptible tomato genotypes as compared to moderately resistant and resistant genotypes. Maximum number of eggs per egg masses was obtained in three highly susceptible genotypes *viz*., Arka Abha (290.00), IC 249503 (285.66) and LE 812 (282.33) whereas minimum number of eggs per egg mass were observed in the resistant genotypes *viz*., Hisar Lalit (64.66), PNR 7 (90.00), HN 2 (92.33) and IIHR 2614 (92.33) and IIHR 2868 (101.33).

Significant differences were noticed among tomato genotypes in decline of top and root growth and increase of J_2 population in *M. incognita* infested soils at harvest after 60 days of transplantation. The extent of reduction in plant growth of tomato genotypes inflicted by nematodes was directly proportionate to increase in reproduction potential of *M. incognita* on specific tomato cultivar.

Tomato cultivar Arka Abha, IC 249503 and LE 812 supported significantly greater number of J_2 and were able to cause more root length and root dry weight reduction compared to all other tomato genotypes. Both nematode population and damaged inflected to plant growth *viz.,* root length and root dry weight in nine tomato genotypes including IIHR 550-3 (32.53 – 4.12), IIHR 915 (31.56 – 4.00), IC 249505 (26.50 – 3.10), IC 249515 (25.00 – 2.20), IC 550742 (25.93 – 2.45), IC 567277 (25.65 – 2.40) and IC 567307 (25.50 – 2.23) were found to be moderately resistant. There was significantly low nematode population coupled with less plant growth reduction *i.e.,* root length and root dry weight were found in Hisar Lalit (40.50 – 5.35), HN 2 (36.70 – 4.36), PNR 7 (40.33 – 5.06), IIHR 2614 (35.33 – 4.35) and IIHR 2868 (35.20 – 4.25) tomato genotypes.

Plant growth reduction in tomato genotypes might be due to severe root galling and arrested root system by nematode infection. The ability of galled roots lead to modification in absorption of water and nutrient from soil and their translocation to foliage resulting in foliage chlorosis and stunting of vegetative growth (Bala, 1984). The arrested root system could not be able to fully explore the soil for water and nutrients (Clark *et al.,* 2003).

The occurrence of variation in susceptibility among forty tomato genotypes to *M. incognita* might be due to genetic differences (Brow *et al.,* 1997; Jacquet *et al.,* 2005). The highly susceptible genotypes supported greatest number of juveniles penetrated and completed their development to maturity as shown by high gall index, more number of females, egg masses and eggs with high reduction in root length and root dry weight present while in resistant cultivar limited numbers of juveniles were able to penetrate, develop to maturity and lay egg masses.

This investigation on the reaction of commercially available tomato genotypes to *M. incognita* provides evidence that they are susceptible to nematodes in the infected field. The compatible reaction of moderately resistant, susceptible and highly susceptible tomato genotypes to *M. incognita* infection indicated that they lack resistant genes so genotypes were unable to stop the penetration, development, and reproduction. This suggests that we need to transfer resistant genes to our tomato genotypes from germplasm to avoid the infection by nematodes, which is essential for the management of root knot nematodes.

In conclusion, this study indicated that significant differences were noticed among the different genotypes against the root knot nematode. The genotypes Hisar Lalit, HN 2, PNR 7, IIHR 2614 and IIHR 2868 were found to be resistant to root knot nematode (*Meloidogyne incognita*). So, these genotypes are promising materials to be used as resistant to root knot nematode. Cultivation of these root knot nematode resistant genotypes will be a profitable alternative for the production of healthy, toxic free tomato to the consumers. However, further study is needed to know the cross compatibility between root knot nematode resistant genotypes with high yielding tomato.

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