IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
Volume **9** | Issue 1 | January, 2014 | 117-122

RESEARCH ARTICLE

Response of wild *solanum* rootstocks to root-knot nematode (*Meloidogyne incognita* Kofoid and White)

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SUMMARY

A study was carried out under glass house condition at the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore during 2012-2013 to identify resistant rootstocks of *Solanum* species for grafting of tomato against root knot nematode, *Meloidogyne incognita*. Seven wild *solanum* rootstocks and one *Physalis* wild rootstock and two tomato F_1 hybrids were screened against the root knot nematode. The experiment was conducted in a completely randomized block design with three replications. The seedlings of the wild rootstocks and tomato hybrids were maintained in pots filled with sterilized soil under glasshouse condition and inoculated with *Meloidogyne incognita* @ two second stage juveniles per gram of soil after 15 days of planting. Sixty days after inoculation, the plants were evaluated for shoot length, root length, shoot fresh and dry weight as well as root fresh and dry weight, number of galls per 10 gram of root, egg mass and females per gram of root, root knot index, soil nematode population per 200 cc of soil and reproduction factor. Less number of galls and egg masses were observed in *Solanum sisymbrifolium* followed by *Physalis peruviana* and *Solanum torvum* rootstocks exhibited resistant reaction. Among tomato F_1 hybrids, TNAU tomato hybrid CO-3 showed moderately resistant reaction. *Solanum violaceum* and tomato hybrid US-618 were highly susceptible to *M. incognita*.

Key Words : *Solanum* rootstocks, Tomato, Root knot nematode resistance, *Meloidogyne incognita*

How to cite this article : Dhivya, R., Sadasakthi, A. and Sivakumar, M. (2014). Response of wild *solanum* rootstocks to root-knot nematode (*Meloidogyne incognita* Kofoid and White). *Internat. J. Plant Sci.*, **9** (1): 117-122.

Article chronicle : Received : 21.09.2013; **Revised :** 15.10.2013; **Accepted :** 01.11.2013

Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetables in India. The incidence of root knot nematode was observed to be high in areas where tomato is cultivated intensively. Root knot nematodes (*Meloidogyne* spp.) have been recorded to cause yield losses ranging from 39.7 to 46.92 per cent in tomato production (Darekar and Mhase, 1988). Khan *et al.* (2000) listed several nematodes that are associated with vegetable crops. Among them the most serious one which cause more damage was *M. incognita*. The disease is expressed by gall formation in the root system and ultimately the plant becomes weak due to interruption in nutrient uptake from the soil and at severe infection the plants may die.

Management of root knot nematodes is difficult as they have a wide host range. Soil fumigants, contact-systemic nematicides and resistant varieties are commonly employed to control root knot nematodes. Soil fumigants such as methyl-bromide were very effective against nematodes, but these chemicals carry environmental risk and also it was banned in India. Hence, there is an urgent need to develop an alternate method to overcome these problems under field condition. Grafting on suitable rootstocks improves the resistance to soil-borne diseases *viz*., Fusarium wilt, bacterial wilt, Verticillium wilt and root knot nematode (Bersi, 2002; Augustin *et al*., 2002). Grafting as an alternative to methyl bromide in field production (Freeman *et al*., 2009); and the use of resistant

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rootstocks for controlling root knot nematode and soil borne diseases such as bacterial wilt [caused by *Ralstonia solanacearum* (Smith)], Fusarium wilt, and southern blight (caused by *Sclerotium rolfsii* Sacc.) (Bausher, 2009; Rivard and Louws, 2008; Rivard *et al*., 2010). To strengthen grafting technology, selection of rootstocks resistant to root knot nematode disease is urgently necessary. Therefore, the present investigation was undertaken to identify resistant wild *solanum* species as rootstocks against root knot nematode.

MATERIAL AND METHODS

Pot culture experiment was conducted under glasshouse condition at the Department of Nematology, TNAU, Coimbatore during 2012-2013. The wild *solanum* species *viz*., *Solanum torvum*, *S. xanthocarpum*, *S. incanum*, *S. aethiopicum*, *S. sisymbrifolium*, *S. viarum*, *S. violaceum* and *Physalis peruviana* and Tomato F_1 hybrids, TNAU Tomato Hybrid CO-3 and US-618 were used in this study. Seedlings were raised in protrays and then transplanted into pots for artificial inoculation. Thirty day- old healthy seedlings were planted in earthen pots containing two and half kilogram of sterilized pot mixture (Red soil: Sand: FYM in 2:2:1 ratio). The experiment was laid out in a Completely Randomized Design with three replications.

Root knot nematode infected tomato plant roots were collected from farmer's field, Coimbatore. Then these roots are confirmed as infected by *M. incognita* under stereoscopic microscope after thorough washing with tap water and staining with acid fuchsin lactophenol. Highly susceptible tomato cultivar PKM-1 was used for developing pure culture of root knot nematode. Plants of tomato (PKM-1) were raised in the pots filled with steam sterilized loamy soil mixed with fine river sand. The potted plants were inoculated with J_2 stage of *M. incognita* @ 2-3 per pot and maintained as pure culture.

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 and freeing the eggs from the egg mass and incubated for 48 hours under laboratory condition. The nematode concentration was adjusted to a known number by addition of water for inoculation. The eggs were kept in Petridishes and frequently aerated with the use of aerator to enable hatching. The nematode inoculum (J_2) was placed in 2cm depth near the 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 in soil, roots 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*., shoot length, root length, shoot fresh and dry weight as well as root fresh and dry weight were measured. Dry weight was determined after drying the plants in a hot air oven at 60^0 C for 72 hours. From the fresh root sample, number of galls per 10 gram of root, egg masses and females per gram of root were counted under stereoscopic microscope after staining with acid fuchsin lacto phenol. The population of *M. incognita* in soil and roots were assessed by using cobb's sieving and decanting method followed by modified Baermann funnel technique (Cobb, 1918; Schindler, 1961). The final nematode population (Pf) was calculated as total number of nematode extracted from both roots and soils. The reproduction factor (Rf) of the nematode in the different plants were obtainted by dividing the final population densities by the initial population densities (Rf= Pf/Pi). The data from the experiments were analyzed statistically following Panse and Sukatme (1967).

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)

Assessment of disease resistance (Root gall indexing):

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

RESULTS AND DISCUSSION

Among the rootstocks, significant difference was observed on plant growth parameters. The highest shoot length, shoot fresh and dry weight were found in *S. sisymbrifolium* rootstock (92.83 cm, 103.87 g and 10.44 g, respectively) followed by *S. torvum* (73.87 cm, 93.53 g and 10.18 g, respectively) and *Physalis peruviana* (Table 1). In slight controversy, the resistant species *S. torvum* had registered the highest root length with value of 44.93 cm; root fresh and dry weight with a value of 54.17 g and 5.53 g, respectively followed by *S.sisymbrifolium* compared to other wild species and tomato hybrids. This might be due to the low nematode reproduction on *S. sisymbrifolium* and *S. torvum* roots. Binks and Gowen (1997) also observed higher root weight with more primary roots in resistant cultivars compared to susceptible cultivars. Thus, root number and size are likely to be significant factors in plants tolerant to nematodes. Good root development potentially favours resistance to plant. In the present study also, the resistant or tolerant species *S. torvum* and *S. sisymbrifolium* showed high number of roots with better root length.

Among the wild species and tomato hybrids, the lowest values for shoot length (19.97 cm), root length (9.10 cm), shoot fresh and dry weight (14.47 g and 1.35 g, respectively) and root fresh and dry weight (7.13 g and 0.68 g, respectively) were noticed in the US-618 tomato. This might probably due to the development of giant cells which cause chocking of xylem vessels and interfere with the nutrient uptake. Secondary effects include reduced photosynthetic efficiency with a reduction in light interception and carbohydrate synthesis. It is in line with Serfoji *et al.* (2010). The mechanism involved in the reduction in shoot length of affected plants might be due to larger amount of growth substances, more tryptophan and other amino acids than un-inoculated plants as reported by Setty and Wheeler (1968). Root knot nematode infested plant had a very shallow and knotted root system which would have resulted in impaired plant growth due to reduced nutrient uptake and declined distribution of hormones, minerals and photosynthates as indicated by Darekar and Mhase (1988).

Solanum sisymbrifolium recorded significantly reduced number of galls (4.45 /10 g root) followed by *Physalis peruviana* (4.87 / 10 g root) and *S. torvum* wild species (5.51 / 10 g root) compared to other wild species and tomato F1 hybrids used in this study (Table 2). Number of egg masses and root knot nematode females were found to be the lowest in *S. sisymbrifolium* (1.30 and 1.70 / g of root, respectively) followed by *Physalis peruviana* and *S. torvum*. This is due to the host status for the invading parasite. This results are in similarity with reports by Roberts and May (1986) where they found greater number of females, galls and eggs per plant in susceptible cultivars as compared to moderately resistant cultivars. In pot culture, the build up of *M. incognita* population was assessed taking into account of the soil nematode population at sixty days after inoculation. The lowest soil nematode population was noticed in the species *S. sisymbrifolium* followed by *Physalis peruviana* and *S. torvum*. This might be due to the presence of resistant gene that limits the reproduction of *Meloidogyne incognita* in soil. These findings are supported by Mattos *et al*. (2011) who found that *solanum* species were resistant to *Meloidogyne incognita.*

Table 1 : Reaction of wild *Solanum* species and tomato hybrids to *Meloidogyne incognita*

Rootstock / scion	Shoot length (cm)	Root length (cm)	Shoot weight (g)		Root weight (g)	
			Fresh	Dry	Fresh	Dry
Solanum torvum	73.87	44.93	93.53	10.18	54.17	5.53
Solanum xanthocarpum	40.17	18.17	35.13	3.48	17.07	1.58
Solanum incanum	49.03	23.90	42.90	4.27	21.90	2.25
Solanum aethiopicum	44.23	21.50	41.50	4.19	18.80	1.93
Solanum sisymbrifolium	92.83	29.47	103.87	10.44	45.90	4.75
Solanum viarum	25.97	14.70	24.13	2.17	12.83	1.17
Physalis peruviana	69.67	25.87	91.43	9.24	30.87	3.30
Solanum violaceum	20.30	13.80	17.67	1.43	11.73	1.07
TNAU tomato hybrid CO-3	24.73	12.67	28.10	2.68	16.20	1.55
US-618	19.97	9.10	14.47	1.35	7.13	0.68
S.E.±	1.14	0.60	0.77	0.63	0.57	0.41
C.D. (P=0.05)	2.38	1.25	1.61	1.31	1.20	0.86

Table 2 : Reaction of wild *Solanum* species and tomato hybrids to *Meloidogyne incognita*

Rootstock / scion	No. of galls / 10 g root	No. of egg masses / g root	No. of females / g root
Solanum torvum	5.51	1.87	2.10
Solanum xanthocarpum	44.57	6.83	31.63
Solanum incanum	23.53	4.23	14.77
Solanum aethiopicum	25.80	4.70	17.83
Solanum sisymbrifolium	4.45	1.30	1.70
Solanum viarum	57.23	8.23	44.90
Physalis peruviana	4.87	1.43	1.87
Solanum violaceum	108.83	30.60	90.33
TNAU Tomato hybrid CO-3	28.67	5.57	22.10
US-618	113.60	32.90	96.17
S.E.±	1.03	0.64	1.26
C.D. (P=0.05)	2.16	1.32	2.62

The suitability of the host for plant parasitic nematode expressed as the ability of the nematode to multiply in the plant and is measured by reproduction factor (Rf) which is the ratio of the number of nematodes recovered at the end of the experiment (Pf) to the number of nematode units used to inoculate the plants (Pi) (Liebanas and Castillo, 2004). A wide range of variation was recorded in reproduction factor (Rf) which ranged from 0.71 (*S.sisymbrifolium*) to 3.97 (Tomato hybrid US-618). On the basis of nematode reproduction, *S.sisymbrifolium*, *Physalis peruviana* and *S. torvum* were categorized as resistant to root knot nematode which might be due to the presence of resistant gene that limits the reproduction of *Meloidogyne incognita* in soil. Similar results were reported by Mattos *et al*. (2011) who found that *S. asperolanatum*, *S. stramonifolium* and *Solanum* sp. were resistant to *M. incognita* race 1 with reproduction factors of 0.17, 0.06 and 0.49, respectively, these values were less than 1. Formation of fewer galls in resistant wild species was probably due to failure of nematode juveniles to produce functional feeding site in the host after invasion and to develop subsequently as reproducing females (Sobezak *et al*., 2005). Biochemical mechanism of invasion supports this mechanism which occurs due to non co-operative action of host tissue or cells. The chemical inhibitors in the host tissue counteract or neutralize the giant cell inducing effect of salivary secretions of the nematode (Barrons, 1939). The rootstocks and scions susceptible to nematode infection supporting the highest population compared to resistant rootstocks.

The resistance or susceptibility of wild species to root knot nematode is assessed by various methods and one such method is root knot indexing. Index based on number of galls is also one of the methods of scoring resistance to root knot nematode. It was revealed from data that among the eight wild species *S.sisymbrifolium*, *Physalis peruviana* and *S. torvum* gave root-knot index 2 with resistant reaction while *S. incanum* and *S.aethiopicum* were moderately resistant against root-knot nematode with root-knot index 3, *S. xanthocarpum* and *S. viarum* showed susceptible reaction and *S.violaceum* gave highly susceptible reaction (Table 3). Among the two tomato F1 hybrids, TNAU tomato hybrid CO-3 gave moderately resistant and US-618 showed highly susceptible reaction. Resistant reaction was observed in wild species like *S.sisymbrifolium*, *Physalis peruviana* and *S.torvum* might be due to the presence of nematode resistant gene. This finding is in line with the reports of Hadisoeganda and Sasser (1982) and Roberts and May (1986) who found resistance due to the gene and these genes made the plant less attractive for attack by nematodes. Compatible and incompatible reactions might be due to the presence of resistant genes, which were activated as a result of nematode invasion and some visible reactions could be observed in the plant cells (Williamson, 1999; Davis *et al.*, 2000; Williamson and Kumar, 2006).

Yamakawa and Mochizuki (1978) and Ali *et al*. (1992) also reported *S.torvum* as resistant to root-knot nematode. Rahman *et al*. (2002) also revealed that *S. sisymbrifolium* and *S. torvum* showed resistant reaction against root-knot nematode. The present findings are in similarity with earlier workers, who stated that *S. torvum* and *S. aethiopicum* have been referred to resistant or poor host of *M. incognita* and *M. javanica* (Daunay and Dalmasso, 1985; Matsuzoe *et al.,* 1993). *S. torvum* also provided some tolerance against nematode but not complete resistance (Ioannou, 2001).

Conclusion:

This study indicated that significant difference was exhibited among the different rootstocks and scions against the root knot nematode. The species, *Solanum sisymbrifolium*, *Physalis peruviana* and *Solanum torvum* were resistant to root knot nematode (*Meloidogyne incognita*), *Solanum incanum* and *Solanum aethiopicum* were moderately resistant to *Meloidogyne incognita*. So these species are promising materials to be used as rootstocks for tomato. Thus, there is potential for use as rootstock in cultivated areas infested with the root knot nematode. Grafting of cultivated tomato varieties / hybrids on related soil borne resistant wild species as rootstocks, will be a profitable alternative for the production

Table 3 : Soil nematode population and root knot index for wild *Solanum* species and tomato hybrids to *Meloidogyne incognita*

Rootstock / scion	Soil nematode population (per 200 cc of soil)	Nematode population		Reproduction factor (Rf= Pf/ Pi)	Root knot index	Reaction
		Initial (Pi)	Final (Pf)			
Solanum torvum	42.53	5000	4206.93	0.84	2	R
Solanum xanthocarpum	135.50	5000	8382.82	1.68	4	S
Solanum incanum	81.47	5000	6580.17	1.32	3	MR
Solanum aethiopicum	87.50	5000	6757.24	1.35	3	MR
Solanum sisymbrifolium	39.43	5000	3528.83	0.71	2	R
Solanum viarum	146.40	5000	8896.56	1.78	4	S
Physalis peruviana	40.27	5000	3689.67	0.74	2	R
Solanum violaceum	246.73	5000	18113.59	3.62	5	HS
TNAU Tomato hybrid CO-3	89.47	5000	7236.45	1.44	3	MR
US-618	252.47	5000	19830.79	3.97	5	HS

R - Resistant MR - Moderately resistant S - Susceptible HS - Highly susceptible of healthy, toxic free tomato to the consumers. However, further study has been needed concerning graft compatibility between root knot nematode resistant rootstocks with tomato.

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