

## Susceptibility Assessments of Tomato Genotypes to Root-Knot Nematodes, *Meloidogyne javanica*

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Root-knot nematodes, *Meloidogyne spp.*, are one of the important plant parasitic nematodes of tomato in the world. The most suitable control method of plant parasitic nematodes is the use of resistance sources and tolerant cultivars. In the earlier studies, the results showed that only 2% (19 out of 537 varieties) were resistant and tolerant to the root knot nematodes. In the supplementary studies, the susceptibility of these 19 tomato cultivars were reassessed again, against *M. javanica*, in the two completely randomized design experiments in the greenhouse and field conditions for the two continuous years. The tomato plants were evaluated 70 days after inoculation on the basis of the gall indexes (GI), final populations (Pf), reproduction factors (Rf) and the root and or the stem weights. The results showed that, the cultivars No. 136 and 109 with GI=2, Rf=4.68 and GI=2.25, Rf=28.4 are tolerant cultivars to the nematode, *M. javanica* in the greenhouse respectively. Also, the cultivar No. 100 was considered to be a susceptible one, with GI= 3.25 and Rf= 0.97. Whereas, in the field conditions, the cultivars 136 and 109 proved to be tolerant with GI, 1.9 and 1.6 respectively.

Abstract

**Keywords:** Cultivars, Resistance, Root- knot nematode, Tomato, Susceptibility.

## INTRODUCTION

Tomato (*Lycopersicon esculentum* Miller, 1768) is one of the vegetable crops, which is used in various ways, such as souse and or etc. Tomatoes are planted in 110,229 hectares in Iran and have 27 tone/hectare average yields. Root-knot nematodes, *Meloidogyne* spp., are the most important tomato parasitic nematodes in Iran and the world. Seven species and five races of these nematodes have already been identified in Iran. *M. javanica* is the most and well distributed one in tomato and other field crops (Akhiyani *et al.*, 1984; Mehdikhani *et al.*, 2003; Janar Dhanan, 2002; Mojtahedi and Santo, 1994; Razaz Hashemi, 2005; Razaz Hashemi, 2006). The infection of the tomato cultivar, Red Cloud VF was reported to be 37% with 20 eggs and larva, as the primary inoculums per gram of soil, in the micro plot in Isfahan. Resistance and susceptible cultivars, offer effective control against these nematodes (Webster, 1972). So, different cultivars of tomatoes were offered to farmer in different countries. Gold set, Nematex and Small Early cultivars were reported as resistant sources to *M. incognita* in Canada. In USA VFN-8, Rossel, Patirot, Healani, Atkinson, Anaha, Nemed were resistant cultivars to *M. incognita* and *M. javanica* (Taylor and Sasser, 1978). In Italy Roma VFN, VFN-77-177-1, VFN-77-92-2, Stumae Ronita were resistant cultivars to root knot nematode (Vito and Lamberti, 1976). In Egypt, Small Early VFN-8 and Ronita were reported as resistant cultivars to root knot nematodes (Akhiyani and Mortazavi, 1992). In India, Pusa-120 and CLL 303-BCI were reported as resistant cultivars to *M. javanica* and race 1 to 4 of *M. incognita* (Prasad *et al.*, 1964). Pelican cultivar was resistant to race 1, 2 and 4 and was susceptible to race 3 of *M. incognita* (Rao *et al.*, 1975). Bush-VFN and VFN-8 were resistant to all races of *M. incognita* and *M. javanica* (Singh and Choudhury, 1974). Kaur *et al.* (1994) studied the reaction of 25 F1 generation hybrid of tomato, which were resistant to *M. incognita* in Ludhiana area in India. Hybrid cultivars which were gained from Castly Roch\* 1792 and Ronita \* Rio Grande were highly resistant, Ronita\* F24-C8 and EC 119192\* KF15 was resistant too. Hybrid of Rio Grand \* Ronita and Pujab Chuhara\* Ronita were moderately resistance to *M. incognita*. Akhiyani (1981) examined 72 seeds from 1982 to find resistant or moderately resistant tomatoes during 1982-1986. Akhiyani selected 19 lines and collected seed. Also, 91 tomato cultivars were sent by Gene bank. Akhiyani and Mortazavi (1992), evaluated 537 cultivars of tomato in order to find resistant cultivars to *M. javanica* based on international project standard of root knot nematodes. Out of these cultivars, 98 % were susceptible, and also some of the cultivars, which were reported as resistant to *M. javanica*, were found to be susceptible. Out of 11 cultivars in final tests, 7 cultivars were determined to be resistant and others were susceptible.

In the present studies, the reaction of 19 tomato cultivars with the high quantity and quality properties were evaluated against *M. javanica* in comparison to the controls, for the two continuous years in the green house and the field conditions.

## MATERIALS AND METHODS

### Evaluation of the cultivars reaction to nematode in the greenhouse conditions

The 20 tomato cultivars were planted in plastic pots filled with 1.5 lit of soils, with the mixture of sand and pasteurized peat with 1:2:1 ratio. Cultivars reaction to *M. javanica* was rated on each tomato plant, infected with the numbers of 5000 eggs and larva as the primary inoculum. Pots were placed in greenhouse at about 25°C for 70 days. Plants were uprooted and gall indexes, number of nematodes per root and soil, reproduction factors and growth conditions of the plant, including root and stem weights were assessed in the complete block designs, indicating 20 treatments and 4 replicates each. The average means were compared based on Duncan Multiple Test Ranges (DMRT).

### **Planting tomato seeds in micro plots**

Tomato seeds were planted in the micro plots, and after rising of the seedlings, the even size and strong ones were transferred in to the field.

### **Selecting infested field to nematode**

The fields which were highly infested to *M. javanica* with the mean numbers of 30 eggs and larva per gram of soil selected in the regions, Vilashahr, Najafabad, in Isfahan province, which then, divided into plots, according to the numbers of treatments and replicates.

### **Evaluation of tomato cultivars reaction to nematode in infested fields**

Tomato seedlings were transferred into the infected fields. Sampling was done from 0 to 30 cm depths in order to determine the primary inoculum of nematodes. After, 4 months, all the plants, 30 plants from each replicates were uprooted and assessed based on 0 to 5 scoring scales (Tayler and Sasser, 1978). Nematode numbers in the roots and the soils from every plot were calculated, in order to determine reproductive factor (RF) (Oostenbrink, 1966). Evaluation of resistance, tolerance, susceptible and hypersensitive reactions of the tomato cultivars were assessed based on Canto-Sanz (1983) method. Tolerance cultivars with the  $RF > 1$  and  $GI < 2$ , the resistant ones  $RF < 1$  and  $GI < 2$ , susceptible cultivars  $GI > 2$  and  $RF > 1$  and hypersensitive  $RF < 1$  and  $GI > 2$  (Canto-Saenz, 1983).

### **Statistical analysis**

The experiments were based on the complete block designs with 20 treatments, tomato cultivars and 4 replicates each. Average mean comparisons were performed based on Duncan tests. Statistical analysis of variance was done with MSTATC computer software (SAS Institute. 1996).

## **RESULTS**

### **Evaluation of tomato cultivars reaction to nematode in greenhouse conditions**

The means of gall indexes, nematode populations, reproduction factors, root and stem weights of the 20 tomato cultivars to the root knot nematodes have been presented in table 1 and 2, along with the means of statistical grouping, based on Duncan tests.

The means of gall indexes showed 9 different significant groups. Variations in gall indexes were between 2 (cultivar No. 134) to 5 (control). Dornus X New gaeker and cultivars No. 20, 14, 109 and 178 had gall indexes of 2.5, 2.75, 2, 2.25 and 2.75, respectively. Control cultivar had gall index 5 (Table 1). Cormello T.M.V.F.N.S. X Tina, Delta X Chef, Delta X Chef and 140 had gall indexes of over 4. So, these results indicating that, these cultivars are susceptible to the root knot nematodes in these experiments (Table 1).

The nematode populations in the roots and related soils of the 20 tomato cultivar were divided into the six distinct and significant statistical groups. Cultivar No. 100 had the least egg and second stage of larvae populations. Delta X Chef cultivar had the maximum No. of eggs and second stage larvae populations. Delta X Chef and Cormello T.M.V.F.N.S. X Tina had more nematode populations in comparison to control treatments (Table 1). Means of nematode reproductions also, showed five significant statistical groups. Cultivar No. 100, Dornus X New gaeker, cultivar No. 136 and Dornus X New gaeker had RF 0.97, 2.32, 4.68 and 4.96, respectively. Cultivar No. 100 had  $RF < 1$ . This scale in Delta X Chef, Cormello T.M.V.F.N.S. X Tina and cultivar No. 140 was more than control treatment. So, these results showed that, these cultivars are highly susceptible to these nematodes.

Means of root weights showed six variable and different statistical groups. Control and cultivar No. 182, 26 had the most root weights respectively. Whereas, the means of stem weights

divided the cultivars into eight distinct and significantly different statistical groups. Cultivars No. 182, 27 and 136 had the maximum stem weights respectively (Table1).

### **Evaluation of tomato cultivars reaction to nematode in infested fields**

Gall indexes, nematode populations per root and soil and reproduction factors of 20 tomato cultivars with the treatments and replications in the field have already been summarized in table 2. Variance analysis showed that, here also the tomato cultivars are significantly different. And, there are differences when, the cultivars are compared with each others and or with the controls. Mean comparison of the gall indexes, nematode populations per root and soil and reproduction factors of the tomato cultivars root and statistical grouping of the means are also presented, based on Duncan tests. Here, the mean comparison of the gall indexes showed nine different group and the range of variations in the gall index was 1.6 (cultivar No. 109) and 4.25 (control). Cultivar No. 109 and 136 had the least gall index, 1.6 and 1.9 respectively, whereas control had gall index 4.25 (Table 2).

Mean comparison of reproduction factors showed 4 different and significant groups. Cultivar No. 136 and 26 had the least reproduction factors in comparison to control which had the most reproduction factors (Table 2).

Reaction of the cultivar No. 100, 136 and 109 in greenhouse and field conditions show that, the cultivar No. 100 was highly susceptible in greenhouse and susceptible in field. Cultivar No. 136 was tolerance in greenhouse and field. Cultivar No. 109 was susceptible in greenhouse and tolerance in field.

### **DISCUSSION**

Resistance in tomato cultivars to root-knot nematodes has the same genetic origins and is controlled by a dominant gene, Mi, which located on the chromosome No. 6 (Harada, 1996; Gilbert and McGurive, 1956; Liharska, 1998). This gene was transferred from the wild tomato (*L. peruvianum*) to some commercial cultivars, and it can be used efficiently against *M. incognita*, *M. arenaria* and *M. javanica* (Canto-Saenz, 1983; Canto-Saenz, 1985, Fassuliotis, 1979). In this study, without considering, Mi genes in view, the reactions of 19 tomato cultivars were evaluated against *M. javanica* in which, the different characters, such as the potentiality of the reproduction factors were taken into accounts by several workers (Khan and Khan, 1991; Oostenbrink, 1966). Here also, the potentiality of the reproduction factors in the tomato cultivars were compared with the potentiality of the reproduction factors in susceptible ones (Taylor, 1967) and also, egg mass indexes and or gall indexes (Taylor and Sasser, 1978), complexes of nematode reproductions and crop losses were taken into considerations (Canto-Saenz 1983; Castagnon-Sereno *et al.*, 1994) . Based on recent factors in greenhouse tests, about 85% of the cultivars were susceptible to nematode with  $GI > 2$  and  $R > 1$  indexes. Cultivar No.136 with  $GI= 2$  and  $R= 4.68$  was tolerant and cultivar No. 100 with  $GI= 3.25$  and  $R= 0.97$  was hypersensitive. In this cultivar  $RF < 1$  and  $GI > 2$ , indicating that, the nematode arrives to the tomato root system, but the resistance of the host prevents the reproductively of the nematodes. Cultivar No. 109 (wild tomato) was comparatively tolerant (Table 1). Cultivars 136 and 109 were introduced tolerant cultivar in the field conditions. About 85-90% of the tomato cultivars were susceptible to *M. javanica*, which is unlike the Akhiyani's report, that they were tolerant and resistant cultivars. Probably different factors had already interfered in this case. Nematode populations influenced the decrease in resistance of the tomato cultivars to root knot nematodes. Economic threshold level of root knot nematode is 0.005-2 egg and larvae per gr of soil (Araujo *et al.*, 1982; Barker, 1976; Ferris, 1978). In the field experiments, the limitations to select the nematode infested soil could be a factor, because the initial population's density of nematode was 30 eggs and larvae per gr soil. Therefore, reproduction in the large scale caused the

breaking of the resistance in some cultivars. Cultivar No. 109 was susceptible in greenhouse and tolerant in the field conditions. This cultivar is a wild one, and it grows with a well developed root in the field. So, this character having a great affect on the reaction of this cultivar (Hashemi and Winstead, 1959).

Cultivar No. 136, which was tolerant to *M. javanica* in the field and greenhouse conditions, could be planted in some regions where, the temperatures reach to and or below 28°C. Usually, in these regions tomatoes are planted in winter and fall where, the temperatures are less than 28°C (Araujo, *et al.*, 1982; Dropkin, 1969; Netscher, 1977). Cultivar No. 136 can be introduced as a tolerant cultivar.

Involving mechanisms in resistant plants could be the production of toxic from the root exudates, the lack of an attractant or the hatching factor in the exudates, a barrier for penetration or the failure of nematodes to develop within plant tissues, the production of lignin and synthesis toxin including phytoalexins (Jenkins and Taylor, 1967; Favery *et al.*, 2001; Jaubert *et al.*, 2002).

Ascorbic acid is generally considered to provide resistance in plants to various pathogens. Low levels of ascorbic acid in tomato cultivars were associated with their susceptibility to *M. incognita* attack. The results showed that, ascorbic acid increase production of hydroxy prolin which lead to increase activity of resistant respiratory Cyanide (Arrigoni *et al.*, 1979; Brueske, 1980). All evidences have shown that, the root cells of resistant plant react against nematode via increase in NADPH oxidase activity. The production of superoxidase in plant cells directly or indirectly may cause the death in hypersensitive cells and as a subsequent to these reactions, establish resistance in plants. Previous studies have indicated that, respiratory resistant cyanide and superoxidase induce the phytoalexin synthesis, then establish resistance in plant during infection process (Favery *et al.*, 2001; Semblat *et al.*, 2001; Semblat and Castagnone-Sereno, 2001).

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## Tables

Table 1. Means of gall index, nematode population, reproduction factor of *M. javanica*, root and stem weight of tomato in greenhouse conditions

Treatment <sup>1</sup>	Gall index	Nematode population per g. of root & soil	Reproduction factor <sup>2</sup>	Stem weight (g)	Root weight (g)	Reaction
Ricraude X SP-100	3.75abcde <sup>3</sup>	234920c	46.98abc	43.75de	20bc	Susceptible
Delta X Chef	4.75ab	246875c	49.37abc	49cde	19.5bc	Susceptible
Delta X Chef	4.25ab	552257a	107.95a	46.75cde	22.25bc	Susceptible
Cormello T.M.V.F.N.S.X Tina	4.5abc	537960a	107.59a	60abcde	31ab	Susceptible
SP-100 X Castlerd (1-13)	3.5abcd	127430d	25.48c	63.5abcde	14.5c	Susceptible
Delta X Chef	3.75abcde	123951d	24.79c	64.5abcde	25.25abc	Susceptible
Dornus X New geaker	3.5abcde	24826e	4.96c	42.75de	20.5bc	Susceptible
Dornus X New geaker	2.5de	11257e	2.32c	43.25de	12.75c	Susceptible
Dornus X New geaker	3bcde	72743de	14.45c	40.5e	23.5bc	Susceptible
20	2.75cde	67144de	12.93c	54.25bcde	18.25bc	Susceptible
26	3.5abcde	123181d	14.23c	79.5ab	26.75abc	Susceptible
66	3.75abcde	124168d	24.83c	63.75abcde	25.5abc	Susceptible
100	3.25abcde	4861e	0.97c	58.25abcde	23bc	Hypersensitive
136	2e	23409e	4.68c	75.5ab	25.75abc	Tolerant
140	4.25abcd	441927b	88.38ab	69abc	25.25abc	Susceptible
170	3bcde	93056de	18.61c	77ab	26abc	Susceptible
109	2.25e	142014d	28.4bc	71abc	22.5bc	Tolerant
178	2.75cde	57625de	15.12c	67abcd	17.5bc	Susceptible
182	3.5abcde	265503c	53.1abc	83a	31.25ab	Susceptible
Control	5a	326389c	65.28abc	67.25abcd	29a	Susceptible

<sup>1</sup> Data are means of four replicates.

<sup>2</sup> Initial population was 500 egg & second stage juveniles.

<sup>3</sup> Means in columns followed by a similar letter are not significantly different at 5% level by DMRT.

Table 2. Means of gall index, nematode population and reproduction factor of *M. javanica* in the field conditions.

Treatment	Gall index	Nematode population per g. of root & soil	Reproduction factor	Reaction
Ricraude X SP-100	3.85ab	4902cd	163c	Susceptible
Delta X Chef	3.9ab	8669bcd	289bc	Susceptible
Delta X Chef	3.45ab	7669bcd	256bc	Susceptible
Cormello T.M.V.F.N.S.X Tina	4.02ab	15458bcd	515bc	Susceptible
SP-100 X Castlerd (1-13)	4.02ab	21745b	724b	Susceptible
Delta X Chef	4.1ab	8943bcd	248bc	Susceptible
Dornus X New geaker	3.92ab	12941bcd	431bc	Susceptible
Dornus X New geaker	3.47abc	16110bcd	537bc	Susceptible
Dornus X New geaker	3.7ab	7651bcd	255bc	Susceptible
20	4.07ab	8775bcd	293bc	Susceptible
26	2.93bcd	2362cd	79c	Susceptible
66	4ab	8061bcd	326bc	Susceptible
100	4.02ab	9626bcd	321bc	Susceptible
136	1.9de	1588d	53c	Tolerant
140	4.25a	23703bc	378bc	Susceptible
170	2.42cde	5088bcd	169c	Susceptible
109	1.6e	3162cd	105c	Tolerant
178	3.52abc	6171bcd	206c	Susceptible
182	3bc	6703bcd	223c	Susceptible
Control	4.25a	38583a	1286a	Susceptible

<sup>1</sup>Data are mean of four replicates.

<sup>2</sup> Initial population was 30 egg & larvae per gr. of soil.

<sup>3</sup> Means in columns followed by a similar letter are not significant at 5% level.



Table 3. Analysis of variance of tomato cultivars to root knot nematodes, *M. javanica*.

Variable sources	CV	SS	MS	F	Prob
Gall index (G.H)	31.37	50.638	2.665	2.59**	0.01
Reproduction factor (G.H)	10.38	85679.79	4509.46	3.16**	0.0005
Nematode. population * (G.H)	89.69	215904.6	113634.7	3.15**	0.001
Root weight	37.83	2755	145	1.83**	0.042
Stem weight	24.41	13358.8	703.09	3.16**	0.0004
Gall index (F)	20.22	45.19	2.38	4.69**	0.005
Repro duction factor (F)	82.76	5789027.7	304685.7	3.78**	0.0001
(F) Nematode. population	24.46	5893.01	31015.1	3.25**	0.0004

G- Green house. F- Field. \*- Nematode population per gr. of root & soil.

- The df, for blocks, treatments and the error are the same for all, i.e. 3, 19 and 52 respectively.

\*\* - Significant at 1% level of probability.

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