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Source of resistance to root-knot nematode (*Meloidogyne javanica*) in tomato cultivars

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Nine tomato cultivars Redstone, Karoon, Falat CH, Falat 111, Efialto, Rutgers, Gina VF, Calj and Mobile were tested for their resistance to root-knot nematodes (*Meloidogyne javanica*) at inoculum levels of 0, 1000, 3000, 5000 juveniles (J2) per pot. Six cultivars found to be susceptible to varying degrees as egg masses were present in all with Rutgers being the most susceptible and also Falat CH, Redstone, Karoon, Mobile, Calj were susceptible while Efialto, Falat 111, Gina VF showed resistant reaction. The inoculums levels had a significant effect ($P < 0.05$) on the number of galls, egg mass, reproduction factor and plant weight. This factors and plant weight was negatively correlated with the highest gall numbers, egg mass, reproduction factor and lowest plant weights recorded at the highest inoculums level in all cultivars except in Efialto, Gina VF, Falat 111 in which there were little variation in gall numbers, egg mass, reproduction factor and plant weights. These cultivars can be used as source of resistance.

Key words: Nematode, *Meloidogyne javanica*, resistance, Tomato.

Introduction

Root-knot nematodes infect a wide range of important crop plants and are particularly damaging to vegetable crops in tropical and subtropical countries. There are more than 90 described species in the genus *Meloidogyne* but the four most commonly occurring species are *Meloidogyne incognita*, *M. hapla*, *M. javanica* and *M. arenaria* (Sasser and Taylor, 1978; Karssen, 2000; Hunt *et al.*, 2005). These species cause galls or root-knots on infected plants. Other symptoms including stunted growth, wilting, and poor fruit yield (Figure 4). Alternatively, integrated nematode management approaches which involve a combination of cultural, chemical and biological methods could more efficiently regulate nematode population (Sikora *et al.*, 2005). An important

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tool and key factor to the success of such control strategies is the careful selection and use of cultivars that suppress nematode population and subsequently yield losses of tomato productions (Molinari, 2011). Local tomato cultivars which currently are excluded from modern large-scale agriculture, are lately gaining popularity (Gomez *et al.,* 2001; Rodriguez-Burruezo *et al.,* 2005; Kumar *et al.,* 2007; Adalid *et al.,* 2010). Due to this trait, local tomato cultivars have been used as a source of resistance genes against pests and disease in breeding programs (Robertson and Labate, 2007). Many tomato cultivars with resistance to the three most widespread species of RKN (*M. javanica, M.incognita* and *M. arenaria*) are commercially available in local markets and used by farmers. These tomato cultivars carry the Mi resistance gene from *Lycopersicon peruvianum* (Fuller *et al.,* 2008), which accounts for a hypersensitive response of the plant. This response results in rapid and localized cell necrosis at the infection site soon after the initiation of nematode feeding and ultimately in the disruption of the nematode life cycle (Roberts, 1992). The resistance mechanism in response to invasion by RKN involves the formation of necrotic cells at the infection site to prevent the juveniles from developing any further. However a high level of genetic variability of RKN has led to the existence of races and virulent populations which can reproduce even on plants carrying the resistance genes (Castagnone- Sereno, 2006). However, there are several reports of resistance breaking root-knot nematode population, virulent against the Mi-gene worldwide (Eddaoudi *et al.,* 1997; Ornat *et al.,* 2001; Tzortzakakis *et al.,* 2005; Devran and Sogut 2010). Evaluation of tomato cultivars resistance to root–knot nematode *Meloidogyne javanica* were reported (Khodayi arbat, 2009;Ahmadi and Mortazavibak, 2004; Moslehi *et al.,* 2010; Saeedi Naini *et al.,* 2004). The present study was conducted to identify the resistance of advanced tomato breeding lines and determine the effects of varying population levels on host plant health being popularized in Khorasan, northeast of Iran.

Material and methods

Inoculum preparation

Collection and isolation of *Meloidogyne* spp. were carried out from naturally infected tomato plants during surveys conducted in 15 major tomato growing regions of khorasan 2009 to 2010. Tomato roots with galls symptomatic of root-knot were collected from the surveyed areas. Samples were placed in plastic bags and transported to the laboratory. At least 10 samples were collected in each field surveyed. Samples collected within a field were pooled as one sample. Fifty egg masses from the same field were selected

and placed beneath the roots of susceptible tomato cv. Rutgers seedlings in 12 cm pots filled with sterile soil. In some cases, soil samples (1000g) were also collected around galled tomato roots in each surveyed field. These subsamples (about 250 $cm^{3)}$ were mixed with an equal volume of pasteurized sands. The resulting mixture was than planted with the susceptible tomato cv. Rutgers in 12 cm diameter pots to allow the development of adult females for identification. Six weeks after inoculation of tomato seedlings, females were extracted from the roots.

Identification and characterization of isolates

Root-knot nematode populations were identified to species and race based on perineal pattern characteristics and differential host tests (Hartman and Sasser 1985). Perineal pattern of mature females were prepared for each root-knot nematode isolate. The root tissues were teased apart with forceps and hair spear to remove adult females. The head and neck region of the nematode was excised and the posterior placed in a solution of 45% lactic acid to remove all body tissues. Then, the perineal pattern was trimmed and transferred to a drop of glycerin and processed as described by (Hartman and Sasser 1985). At least 10 perineal patterns were examined to makes the identification of nematode species of each sample.

Root samples from infected Rutgers tomatoes were immersed in 0.5% sodium hypochlorite and the eggs collected on the 500 mesh sieve were rinsed gently with tap water for 5 min to remove all residual bleach. The differential hosts set included cotton (*Gossypium hirsutum* CV. Delta pine 16), Peanut (*Aracbis hypogaea* CV. Florunner), Pepper (*Capsicum annuum* CV. Early California wonder), Tobacco (*Nicotiana tabacum* CV. NC 95), Watermelon (*Citrullus vulgaris* CV. Charlestone Grey) and tomato CV. Rutgers. These were grown in 12 cm diameter pots filled with sterile clay-loam soil, PH 6.5. Four seedlings (4 to 6 week old) of each differential host were inoculated by pipetting approximately 4000 eggs in 10 ml of water into each pot. Sixty days after inoculation, plants were removed from the pots and stained with Phloxine B (15 mg/liter of tap water) for 20 min. The nematode reproduction was assessed on each plant of the differential set by counting the number of egg masses.

Greenhouse evaluation of tomato breeding lines

Nine tomato breeding lines developed at the agriculture and research center and natural resources of Khorasan Razavi, were evaluated under greenhouse conditions for resistance to *M. javanica*. Tomato breeding lines

(Efialto, Karoon, Falat CH, Falat 111, Mobile, Redstone, Calj, Gina VF, Rutgers) were grown in pots each containing 1kg of autoclaved soil and arranged on greenhouse benches in a randomized complete design with four replications. For protection against early blight and late blight, plants were sprayed at 14-day intervals with metalaxyl plus mancozeb at 1.2 kg/ha. Weed control was done by manual hoeing. At final harvest, all plants were uprooted and the incidence of plants with galled roots was assessed. Resistance tests were carried out in an air conditioned room. Soil temperature was maintained below 28^0C to be sure that any Mi-gene breakdown would not be due to high temperature. Plants irrigated with a nitrogen solution (Coic and Lesaint 1975).

The J2 inoculum was added one day after transplanting at the rate of 0, 1000, 3000, 5000 J2 per pot using 1 ml micropipette into two wells near the roots. Sixty days after inoculation, plants were removed from the pots, and the root systems were washed free of soil and stained with Phloxine B. The reaction of tomato lines, expressed as the egg mass production, was scored as follows: 0 = no egg masses, 1 = 1 to 2 egg masses, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, and 5 = more than 100 egg masses.

Statistics analysis

PROC MIXED, v. 9; SAS Institute, Cary, NC was used to analyze data for experiments. Prior to statistical analyses data were checked for normality and homogeneity of variance to determine treatment effects. Cultivar was classified as a random effect. Fisher's least significant difference (LSD) test was used to determine differences ($P \leq 0.05$) among the cultivars, and mean comparisons were made using Duncan's multiple range test.

Results

Of the tomato lines tested, Efialto, Falat 111 and Gina VF were highly resistant to *M. javanica* and did not develop root-knot symptoms. Mobile, Falat CH, Karoon, Redstone, Calj were less susceptible and Rutgers was most susceptible to *M. javanica*. A direct relationship was observed between the gall number and Egg masses the inoculation level for all cultivars except for Efialto, Gina VF and Falat 111 which had relatively little variation in gall number and plant weight with increasing inoculum level (Figure 1 and Table 2).

The plant weights in all cultivars except Efialto, Gina VF and Falat 111 decreased as compared to the controls with increasing inoculum levels (Figure 2). The greatest percentage reduction in plant weight compared to the control was observed for the cultivar Rutgers while Redstone, Calj, Falat CH, Karoon and Mobile had the least variation in the percentage plant weight loss (Figure

3). The inoculum level was found to have significant (P≤ 0.05) on the number of galls and egg masses and plant weight. The reproduction factor of *M. javanica* on the Karoon, Falat CH, Mobile, Redstone, Calj and Rutgers were significantly different (Table 2).

Table 1. Factor plant growth indices produced by *M. javanica* on nine cultivars inoculated with 1000, 3000, 5000 nematodes. Plant at 60 after inoculation

Index / Cultivar	Shoot fresh weight	Root fresh weight	Root dry weight	Shoot dry Weight	Stem length	Root length	Fruit Weight
Redstone	65.21[b]	16.90[b]	12.96[b]	59.66[a]	36.62[a]	20.68[b]	30.70[b]
Calj	68.34[b]	18.68[b]	15.28[b]	67.30[b]	44.87[b]	21.93[b]	33.80[b]
Mobile	66.18[b]	18.87[b]	12.04[b]	50.40[b]	44.75[b]	22.50[b]	32.15[b]
GinaVF	98.93[a]	7.78[a]	2.20[a]	89.10[a]	53.54[a]	31.37[a]	73.63[a]
Karoon	65.26[b]	17.46[b]	13.30[b]	48.26[b]	41.78[b]	20[b]	30.25[b]
Falat CH	64.12[b]	15.62[b]	12.24[b]	43.71[b]	42.31[b]	22.18[b]	34.06[b]
Falat 111	96.90[a]	4.69[a]	4.50[a]	66.95[a]	52.06[a]	33.56[a]	70.05[a]
Rutgers	45.26[c]	28.34[c]	22.87[c]	35.46[b]	41.62[b]	21.68[b]	10.30[c]
Efialto	100.15[a]	6.84[a]	3.100[a]	64.34[a]	52.46[a]	34.43[b]	73.63[a]

Data are means of four replications. Means in each line, for each index, followed by similar letters are not significantly different using Duncan's multiple range tests ($p \leq 0.05$).

Table 2. Gall and egg mass, reproduction factor indices produced by *M. javanica* on nine cultivars inoculated with 1000, 3000, 5000 nematodes. Plant at 60 after inoculation

Index / Cultivar	Total Gall	Total eggmass	Reproduction factor	Gall index	Eggmass index
Redstone	5.35 [b]	5.74 [b]	1.11 [b]	1.69 [b]	1.59 [b]
Calj	6.90 [b]	6.62 [b]	1.12 [b]	1.67 [b]	1.67 [b]
Mobile	7.09 [b]	7.41 [c]	1.10 [b]	1.74 [b]	1.74 [b]
GinaVF	1.42 [a]	1.95 [a]	0.80 [a]	1.20 [a]	0.20 [a]
Karoon	6.32 [b]	6.90 [b]	1.13 [b]	1.72 [b]	1.74 [b]
Falat CH	5.57 [b]	5.23 [b]	1.11 [b]	1.68 [b]	1.67 [b]
Falat 111	1.53 [a]	1.18 [a]	0.85 [a]	1.21 [a]	0.19 [a]
Rutgers	18.47 [c]	16.33 [c]	1.91 [c]	1.93 [c]	1.93 [c]
Efialto	1.07 [a]	1.93 [a]	0.74 [a]	1.17 [a]	0.08 [a]

Data are means of four replications. Means in each line, for each index, followed by similar letters are not significantly different using Duncan's multiple range tests ($p \leq 0.05$).

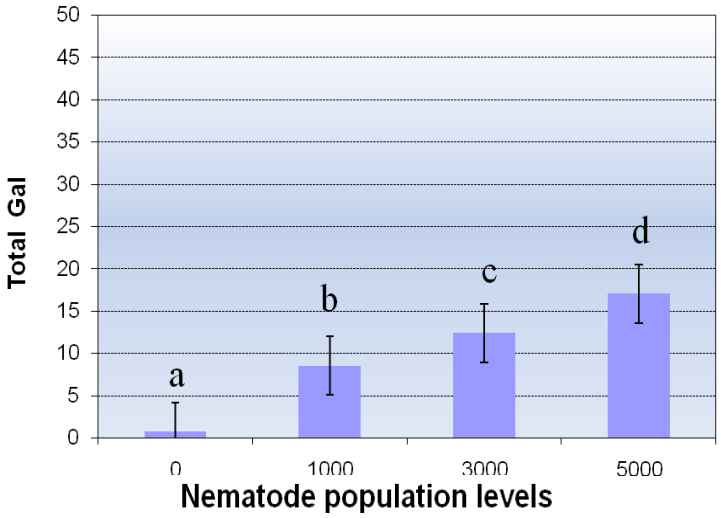

Fig. 1. Effect of inoculums levels on gall number.

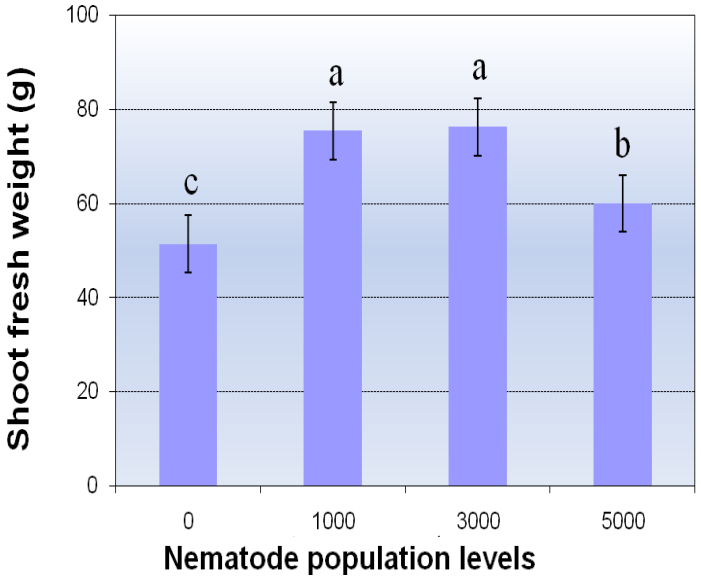

Fig. 2. Effect of inoculums level on plant weight.

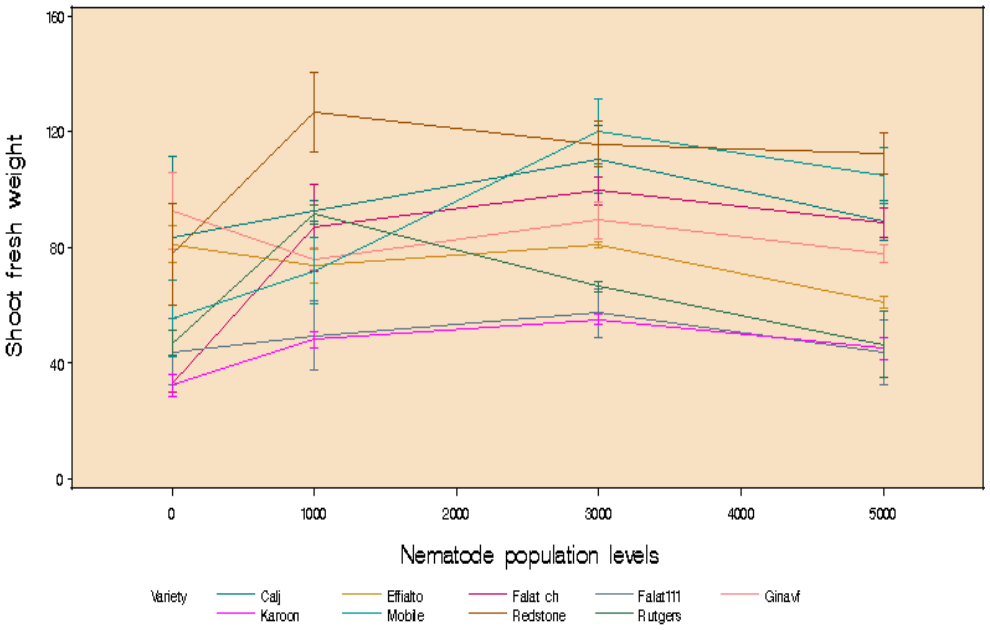

Fig. 3. Percentage plant weight loss vs. inoculum level.

Fig. 4. Comparative symptoms of nematode on resistance and susceptible cultivars (A) Gina VF (5000 nemtodes) and (B) Rutgers (1000 nemtodes).

Discussion

The susceptibility of a plant to RKN depends on the ability of RKN juveniles to penetrate the roots of the plant and cause the formation of giant cells which appears as knot (galls) on the roots (Chen *et al.*, 2004). The juveniles feed and molt twice before developing into the adult stage (Siddiqi,

2000). The adult female RKN stays inside the giant cells and continues to feed and produces egg mass in a gelatinous matrix protruding out of the root gall.

The egg masses give rise to infective juveniles (J2) which may infect other uninfected roots of the same plant or migrate and infect the nearby plants. In case of a plant resistant to RKN, the juveniles are either unable to penetrate the roots, or die after penetration or are unable to complete their development, or females are unable to reproduce. The Mi gene confers resistance by localized tissue necrosis around the region where the juveniles are unable to establish feeding sites resulting in their death or migration out of the roots (Milligan *et al.*, 1998; Lopez-Perez *et al.*, 2005). The evaluation of root galls along with egg mass on all nine varieties of tomato plants indicates that three cultivars of the varieties are resistant to root-knot nematodes. The significant differences in the number of galls present on each of the six varieties indicate different levels of susceptibility. The level of susceptibility is controlled by the presence of the tomato cultivar (Castagnone- Sereno 2006; Jacquet*et al.*, 2005). The homozygous or heterozygous state of the Mi locus has been found to affect the degree of resistance to RKN, with the cultivars having the heterozygous from of the Mi gene being more susceptible than the homozygous cultivars (Jacquet *et al.*, 2005). The variation in the susceptibility to RKN in the Karoon, Falat CH, Redstone, Mobile, Calj, Rutgers tomato cultivars screened is likely to be due to the genetic differences between the cultivars and thus explains the variation in gall numbers and egg masses.

Rutgers was found to be the most susceptible as greatest number of juveniles penetrated and completed their development to maturity as shown by the high gall numbers and egg masses present. Calj, Falat CH, Redstone, Mobile, Karoon were the least susceptibility variety as only a limited number of juveniles were able to penetrate, develop to maturity and lay egg masses. Increasing inoculums levels led to an increase in the number of galls with greater reduction in plant weight for all varieties except for Efialto, Gina VF, Falat 111. This shows that when the inoculums levels are high, greater number of juveniles are able to infect the plant roots which results in reduced nutrient and water uptake by the roots and consequently poor plant growth (Karssen and Moens 2006). In Efialto, Gina VF, Falat 111, even higher inoculums (5000 J2) could not establish a larger population, indicating the presence of some genetic resistance and consequently insignificant decrease in plant weight with increasing inoculums levels. So three of nine cultivars of tomato, used in the present study, Efialto, Gina VF and Falat 111, are cultivars recommended for source of resistance. Further trials with higher inoculums levels or planting in heavily infested soil would give a more conclusive result about the susceptibility of each cultivar and the effect of RKN on yield quantity and

quality. The variability in pathogenicity is also dependent on the genetic variability of RKN population and species composition but since the populations used for inoculation were raised from a single egg mass under the same conditions and host plants it is likely to have had minimal effect in this investigation. There is sophisticated interaction between the host plant and root-knot nematodes and a number of studies have found resistance breaking pathotypes of RKN that are able to parasitize even RKN resistant plants (Jacque *et al.,* 2005; Abad *et al.,* 2003; Baicheva *et al.,* 2002) which is a major limiting factor in using plant nematodes. However, identification and use of RKN resistant and tolerant varieties can still be a viable means of minimizing loss caused by RKN. Another factor which needs to be taken into consideration in any further investigation is the quality and quantity of fruit production of the resistant, less susceptible and highly susceptible varieties because at times the resistant varieties do not produce fruit with the desirable taste and quality (Lopez-Perez *et al.,* 2005).

As conclusion, this study revealed that grafting of desired varieties on the roots of the resistant varieties can be aonsidered as alternative but requires technical knowledge and has additional costs associated with getting the grafted plants to the farmers. The susceptibility of the different tomato varieties has important implication on the yield and economic returns useful to farmers while selecting the variety for planting on RKN infested field.

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