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Response of tomato rootstocks with the *Mi* resistance gene to *Meloidogyne incognita* race 2 at different soil temperatures

Zübeyir DEVRAN[1], Mehmet Ali SÖĞÜT[2] and Nedim MUTLU[3]

[1] Batı Akdeniz Agricultural Research Institute, P.O. Box 35, 07100 Antalya, Turkey
[2] University of Süleyman Demirel, Faculty of Agriculture, Department of Plant Protection, 32260 Isparta, Turkey
[3] Akdeniz University, Seed and Agricultural Biotechnology Center, Antalya, Turkey

Summary. Rootstocks have been effective against many soil-borne pathogens in protected tomato production. Rootstocks with heat-stable root-knot nematode resistance may prolong the production season since the root-knot nematode resistance gene *Mi-1.2* irreversibly breaks down at soil temperatures above 28°C. The objective of this study was to investigate the effect of soil temperature on root-knot nematode resistance conferred by two genes of tomato, using some commercial tomato cultivars, rootstocks, and PI lines. The response of these genes against *Meloidogyne incognita* race 2 was studied in two commonly used rootstock cv. Beaufort and Vigomax, in tomato cultivars Astona RN F1 and Simita F1, and in *Solanum lycopersicum* L. accessions PI126443 and PI270435, known to possess heat-stable nematode resistance, at 24°C and 32°C under controlled conditions. Each plant was inoculated with 1000 *M. incognita* race 2 second-stage juveniles (J2s) and its response was evaluated 8 weeks post inoculation. The presence of the *Mi-1.2* gene was determined with molecular markers. Astona RN F1, Vigomax, Beaufort, PI126443 and PI 270435 which carried the *Mi-1.2* gene were resistant to *Meloidogyne incognita* race 2 at 24°C. The egg masses and J2s were significantly fewer in these lines than in the susceptible Simita F1 at 24°C, and there were no significant differences among resistant plants. In contrast, there were significant differences in the galling index among heat-stable sources and plants containing the *Mi-1.2* gene. Simita F1, Astona RN F1 and the rootstocks had a susceptible reaction to *M. incognita* race 2 at 32°C, but PI 126443 and PI 270435 were resistant. However, at this temperature there were significant differences in the number of juveniles in the soil, the egg mass and the galling index between the heat-stable and the heat-unstable plants.

Key words: *Mi* gene, molecular marker, root-knot nematode, plant resistance.

Introduction

Tomato (*Solanum lycopersicum* L..) is an important crop in Turkey with an annual production of 10 million tons (FAO, 2009). The root-knot nematode (RKN), *Meloidogyne* spp., causes serious yield losses to tomato, especially in greenhouse production. *Meloidogyne incognita*, *M. javanica* and *M. arenaria* are the most common and economically important RKN species in Turkey (Elekçioğlu and Uygun, 1994; Elekçioğlu *et al*., 1994).

Corresponding author: Z. Devran
Fax: +90 242 3466880
E-mail: zubeyird@yahoo.com

Management of RKN is difficult as they have a wide host range. Soil fumigants, contact-systemic nematicides, resistant varieties and rootstocks are commonly employed to control RKN. Soil fumigants such as methyl-bromide were very effective against nematodes, but these chemicals carry an environmental risk. Plant resistance is currently considered as an environmentally friendly alternative method to control soil-borne pathogens. Genetic resistance has been effective against RKN in tomato (Messeguer *et al*., 1991). Cultivated tomato plants are naturally susceptible to RKN, but some accessions of the related tomato species, *S. peruvianum* possess a single dominant gene (*Mi*) that confers resistance to RKN (Medina-Filho and Stevens, 1980; Roberts and Thomason, 1986; Messeguer *et al*., 1991). This resistance gene controls the three

major RKN species *M. incognita*, *M. javanica* and *M. arenaria* (Roberts and Thomason, 1986) in tomato. The *Mi* gene was mapped to the short arm of tomato chromosome 6 (Kaloshian *et al*., 1998). Two homologs of this gene *Mi-1.1* and *Mi-1.2* were identified at the *Mi* locus. However, only *Mi-1.2* conferred resistance to RKN in an experimental assay (Milligan *et al*., 1998). The functional *Mi-1.2* gene is referred to as *Mi* hereafter.

The *Mi* gene controls RKN at soil temperatures below 28°C and has commonly been used in tomatoes grown throughout the world. However, it breaks down irreversibly at soil temperatures above 28°C (Dropkin, 1969). In addition, some nematode populations have overcome the resistance conferred by *Mi* as they have become Mi-virulent (Roberts *et al*., 1990; Castagnone-Sereno, 1994; Kaloshian *et al*., 1996).

Because of linkage drag, tomato cultivars carrying the *Mi* gene sometimes have undesirable horticultural traits such as smaller fruit size. To overcome these negative traits, commercially accepted susceptible lines are grafted onto rootstocks carrying the *Mi* gene. Tomato rootstocks carrying the *Mi* gene effectively control RKN when compared with non-grafted susceptible plants (Lee, 1994; Lopez-Perez *et al*., 2006). Grafting provides some additional advantages, such as greater stress factor tolerance and higher yield (Ioannou, 2001; Lee, 2003). Recently, grafting has become very popular in areas of Turkey where protected vegetables are grown and this technique is also used by tomato growers. However, it is not known what the response of these rootstocks to RKN is at different soil temperatures. The soil temperature in the Mediterranean coastal areas of Turkey in late summer and early fall is high (above 28°C) and this represents a risk for tomato cultivars carrying the Mi gene, as their resistance is known to be broken by a soil temperature over 28°C. In addition, an effective and widely used chemical method to control RKN, methyl bromide, was banned in Turkey at the end of 2007. Therefore, resistant varieties and grafting methods have become important alternatives to control RKN in tomato, since rootstocks have more vigorous root systems than susceptible or resistant cultivated tomato, as they provide enhanced tolerance to nematode infestation. Additional advantages of grafting are that grafted plants have all the agronomic traits of the cultivated varieties, and that it is cheaper than chemical treatment.

The objective of this study was, under controlled conditions, to evaluate the resistance of tomato rootstocks, commercial F1 hybrids, and PI lines to *M. incognita* race 2 at soil temperatures of 24°C and 32°C.

Materials and methods

Plant material

Seeds of *S. lycopersicum* cultivars (Astona RN F1 and Simita F1) and *S. peruvianum* heat-stable genetic plants (PI126443 and PI270435) were provided by seed companies and the İzmir Institute of Technology (İzmir, Turkey) respectively. Seedlings of Beaufort and Vigomax rootstocks were provided by Antalya Tarım (Antalya, Turkey) (Table 1).

Nematode culture

Meloidogyne incognita race 2 was maintained

Table 1. Tomato plants, origin and resistance characteristics.

Plant material	Availability	Seed company/Institute	Root-knot nematode resistance[b]
Astona RN F1	Commercial	Nunhems	*Mi*
Simita F1	Commercial	Nunhems	*mi*
Beaufort	Commercial	De Ruiter Seeds	*Mi*
Vigomax	Commercial	De Ruiter Seeds	*Mi*
PI126443	Experimental	İYTE[a]	*Mi-3*, heat-stable
PI270435	Experimental	İYTE[a]	*Mi-2*, heat-stable

[a] İzmir Institute of Technology, Faculty of Science, Molecular Biology and Genetics, İzmir, Turkey.
[b] Information from the seed company's description. Mi, resistant; mi, susceptible.

on susceptible fresh market tomato plants Tueza F1 (Multi Tarım, Antalya, Turkey).

DNA extraction

DNA was extracted from young leaf tissue using the Promega DNA isolation kit (Promega Corporation, Madison, WI, USA), following manufacturer's instructions.

Genomic DNA amplification

The codominant cleaved amplified polymorphisms (CAPs) marker REX-1 (Williamson *et al.*, 1994) and the codominant gene-specific marker PMi12 (El Mehrach *et al.*, 2005) were used to detect the resistance gene *Mi-1.2*. PCR was carried out in a 25 μL reaction volume containing 10×PCR Buffer (Vivantis, Selangor DE, Malaysia), 0.2 mM dNTP, 0.4 μM of each primer, 2 mM $MgCI_2$, 20 ng of template DNA and 1 Unit Taq DNA Polymerase. The thermocycler was programmed for 3 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 54°C (all primers) and 2 min at 72°C and ended with 7 min incubation at 72°C. Five μL of each reaction was separated by electrophoresis in TAE buffer 1.5% agarose gel to ascertain whether or not the PCR amplification was successful.

PCR products obtained from PMi12 were not digested by any restriction enzyme, but amplification products obtained from the REX-1 marker were digested with *Taq*I restriction enzyme according to manufacturer's instructions. The digestion products were analyzed on a 2.5% agarose gel by electrophoresis in 1×TAE buffer.

Screening tests at soil temperatures of 24°C and 32°C

Tomato seeds were germinated in seed trays containing steam-sterilized sandy soil, and 2-week-old seedlings were transplanted singly to 700 ml plastic pots. Egg masses of *M. incognita* race 2 were collected from the roots using a small needle and were hatched at room temperature (24–25°C). Hatched J2s were counted under a stereomicroscope and plants with four true leaves were inoculated with 1000 2nd stage juveniles each. Plants were maintained at 25°C in a growth chamber, arranged in a randomized block design with 5 replicates. Eight weeks after nematode inoculation the plants were removed and the root systems were carefully washed under tap water.

Egg masses and galls were counted on each root system and the root galling index was scored on each root using a gall index scale from 0 to 10, where 0 represented roots with no galls, and 10 roots with maximum galling at the end of the experiment (Barker, 1985). *M. incognita* race 2 J2s were extracted from 100 g soil with a modified Baermann Funnel (Hooper, 1986) and counted. The reproduction factors (Rf=Pf/Pi) were determined (Ferris and Noling, 1987).

Data analysis

The number of J2/100 g soil (Pf), reproduction factors, the egg mass number, and the root galling indices for each pot were subjected to analysis of variance (ANOVA). The significance of the differences between Simita F1 (susceptible cultivar), Astona RN F1 (resistant cultivar), the rootstocks (Beaufort and Vigomax) and the heat-stable plants PI 126443 and PI 240435 was tested with Duncan's multiple range test at the $P \leq 0.05$ significance level using the SPSS statistical program (SPSS, 12.0, Chicago, IL, USA).

Results

Detection of *Mi-1* gene by molecular markers

PCR with the REX-1 marker yielded a single band of approximately 700 bp and digestion of REX-1 marker PCR products with TaqI in the homozygous resistant rootstocks (Beaufort and Vigomax) and the accessions PI 126443 and PI 270435 yielded

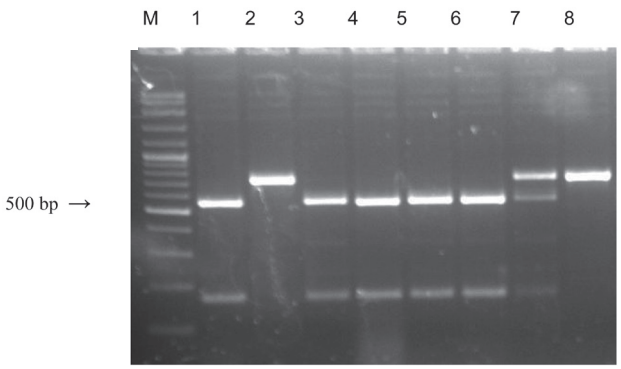

Figure 1. Digestion of REX-1 PCR product with *Taq* I (M, 1 kb molecular weight marker (Vivantis): 1, homozygous control plant; 2, susceptible control plant; 3, Beaufort; 4, Vigomax; 5, PI 126443; 6, PI270435; 7, Astona RN F1; 8, Simita F1.

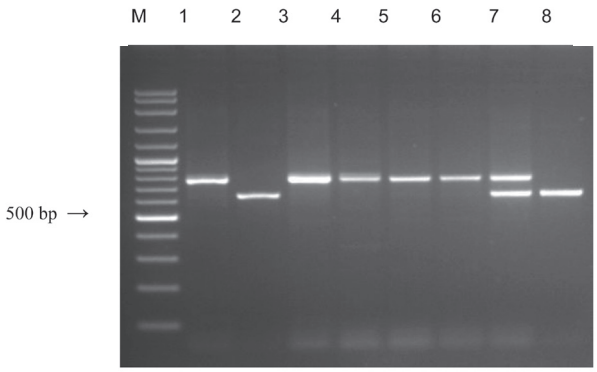

Fig. 2. Amplification profile of PMi12F1 and PMi12R2. M, 1 kb molecular weight marker (Vivantis): 1, homozygous control plant; 2, susceptible control plant; 3, Beaufort; 4, Vigomax; 5, PI 126443; 6, PI 270435; 7, Astona RN F1; 8, Simita F1.

fragments with bands at 550 bp and 150 bp (Figure 1). The homozygous susceptible cultivar Simita F1 yielded a single fragment of 700 bp, while Astona RN F1 had three fragments (700 bp, 550 bp and 150 bp), indicating a heterozygous state (Figure 1).

PCR with the PMi12 primer produced fragments with single 720 bp and 620 bp bands for the homozygous resistant and susceptible cultivars respectively, and two bands (620 bp and 720 bp) for the heterozygous cultivar Astona RN F1. Beaufort, Vigomax, PI 126443 and PI 270435 produced a 720 bp and Simita F1 a 620 bp (Figure 2 and Table 2).

Response of plants to *M. incognita* race 2 at soil temperature of 24°C and 32°C

At 24°C, Simita F1, as expected, had a susceptible reaction to *M. incognita* race 2 and the highest number of egg masses, and galls were detected on infected roots. With this cultivar, the highest number of juveniles was found in the soil at the end of the experiment (Table 3). Simita F1 had Rf factors of 2.6 indicating that this cultivar is an efficient host for *M. incognita* race 2 at 24°C (Table 3). Astona RN F1, Vigomax, Beaufort, PI 126443 and PI 270435 were all significantly ($P \leq 0.05$) resistant at 24°C. The number of egg masses, the root gall index and the number of J2s in the soil was very low but with some differences (Table 3). The root egg masses and the number of juveniles in the soil did not differ significantly ($P \leq 0.05$) between the tomato cultivar Astona RN F1, the rootstocks Vigomax and Beaufort and the heat-stable materials PI 126443 and PI 270435.

At 32°C, Simita F1, and Astona RN F1 had a susceptible reaction to *M. incognita* race 2 as well as the rootstocks Vigomax and Beaufort. In contrast, the accessions PI 126443 and PI 270435 continued to be resistant at this temperature (Table 4). The results clearly show that the rootstocks and Astona RN F1 containing the *Mi-1* gene lost their resistance to *M. incognita* race 2 at 32°C (Table 4). However, the number of J2s, the egg masses and the gall index were significantly lower in all these plants containing the *Mi* gene than in the susceptible plant that lacked the *Mi* gene. Specifically, the number of egg masses in Astona RN F1 was significantly lower than that in the rootstocks but with that cultivar there were more juveniles in the soil than with Vigomax and Beaufort. In contrast, there was no significant difference in the gall index ($P \leq 0.05$). The number of J2s differed very significantly. The two heat-stable resistance accessions, PI126443 and PI 270435 did not differ significantly in the number of their egg masses and the gall index (Table 4).

Table 2. Molecular marker analysis of the Mi gene.

Plant material	Rex-1 marker[a]	PMi12 marker[a]
Astona RN F1	*Mi*/*mi*	*Mi*/*mi*
Simita F1	*mi*/*mi*	*mi*/*mi*
Beaufort	*Mi*/*Mi*	*Mi*/*Mi*
Vigomax	*Mi*/*Mi*	*Mi*/*Mi*
PI126443	*Mi*/*Mi*	*Mi*/*Mi*
PI270435	*Mi*/*Mi*	*Mi*/*Mi*

[a] *Mi*/*Mi*, homozygous resistant; *Mi*/*mi*, heterozygous resistant; *mi*/*mi*, susceptible.

Table 3. Response of *Meloidogyne incognita* race 2 at 24°C soil temperature to tomato rootstocks, resistant and susceptible cultivars, and heat-stable plants.

Plant material	Number of J2s[a] 100 g soil^{-1}	Rf[b]	Number of egg masses	Galling index
Astona RN[c] F1[b]	36.0±22.3 a [g]	0.04±0.02 a	8.2±2.2 a	1.3±0.2 ab
Simita F1[d]	2672.0±373.6	2,67±0.37 b	325.6±24.9 b	5.8±0.4 c
Beaufort[e]	96.0±60.1 a	0.10±0.06 a	13.6±3.5 a	1.9±0.3 b
Vigomax [e]	88.0±49.2 a	0.09±0.05 a	14.4±2.4 a	1.8±0.2 b
PI126443[f]	1.6±0.8 a	0.002±0.001 a	1.2±0.6 a	0.6±0.3 a
PI270435 [f]	0.6±0.4 a	0.001±0.0004 a	0.8±0.4 a	0.6±0.3 a

[a.] J2s, second-stage juveniles.
[b] Rf, reproduction factor.
[c] Resistant cultivar.
[d] Susceptible cultivar.
[e] Rootstocks.
[f] Heat-stable material.
[g] Means in columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Table 4. Response of *Meloidogyne incognita* race 2 at 32°C soil temperature to tomato rootstocks, resistant and susceptible cultivars, and heat stable plants.

Plant material	Number of J2s[a] 100 g soil^{-1}	Rf [b]	Number of egg masses	Galling index
Astona RN[c] F1[b]	1636.0±231.7 b [g]	1.64±0.23 b	72.8±13.6 b	4.0±0.3 b
Simita F1[d]	3952.0±436.7 c	3.95±0.44 c	499.2±37.9 d	6.4±0.4 c
Beaufort[e]	820.0±189.0 ab	0.82±0.19 ab	161.6±25.1 c	4.6±0.3 b
Vigomax[e]	1176.0±449.7 b	1.18±0.45 b	162.4±23.6 c	4.7±0.4 b
PI126443[f]	0.8±0.4 a	0.001±0.0004 a	0.8±0.4 a	0.6±0.3 a
PI270435[f]	0.6±0.4 a	0.001±0.0009 a	0.6±0.3 a	0.6±0.3 a

[a.], [b], [c], [d], [e], [f], [g] See Table 1.

Discussion

The presence of the RKN resistant gene *Mi* in the plants was confirmed with the Rex-1 and PMi12 markers. Marker analysis showed that the rootstocks and the heat-stable plants were homozygous resistant, that Astona F1 was heterozygous, and that Simita F1 was susceptible. Although the Rex-1 marker has been used to transfer resistance to RKN in tomato breeding (Williamson *et al*., 1994; Skupinova *et al*., 2004), El Mehrach *et al*. (2005) reported that this marker could not be used in tomato hybrid lines with introgressions of *S. habrochaites* and *S. chilense*. Therefore the Mi gene-specific marker PMi12 was also used. In the present study, the two markers had the same DNA profiles. According to Cortada *et al*. (2008), the Beaufort cultivar and the hybrids containing the *Mi* gene were homozygous

and heterezygous resistant, respectively, with the Rex-1 marker, which is consistent with the results obtained.

Tomato cultivars with resistance to RKN have recently become commercially available and they represent a valuable resource for nematode management. In these plants, nematodes fail to develop and reproduce normally allowing the plants to grow and produce even though the nematode infects the roots. However, although the *Mi* gene is effective at soil temperatures below 28°C, the resistance it confers breaks down irreversibly above 28°C (Dropkin, 1969). As expected, the commercial hybrid Astona RN F1, the rootstocks and the heat-stable plants were resistant to *M. incognita* race 2 at soil temperature of 24°C. At 32°C, however, only PI126443 and PI 270435 showed complete resistance to *M. incognita* race 2, suggesting that the heat-stable plants possess additional resistance gene(s) effective against *M. incognita* race 2 at the higher temperature. These findings were supported by the nematode reproduction factor (Rf) used as a test to verify host resistance. An Rf of >1 indicates a good host, and an Rf <1 a poor host. Except for Simita F1, the materials tested had an Rf of 0 at 24°C but only the heat-stable plants had an Rf of 0 at 32°C.

The accessions PI126443 and PI 270435 were resistant to *M. incognita* race 2 at 32°C and this is consistent with results reported elsewhere (Cap *et al*., 1993; Yaghoobi *et al*., 1995; Veremis and Roberts, 1996; Veremis *et al*., 1999). It has also been reported that these accessions cannot be practically used in plant breeding programs because of incompatibility barriers with cultivated tomato (Taylor, 1986; Lefrancois *et al*., 1993). However, Doğanlar *et al*. (1997) stated that this incompatibility can be overcome by embryo culture methods. Unfortunately, cultivars with heat-stable resistance to RKN are not currently available.

In conclusion, RKN resistant rootstocks have commonly been used by tomato growers to control RKN. However, heat-stable RKN resistance is still a problem and this needs more attention not only for tomato but for all vegetable crops in the Mediterranean region. Thus, the development of rootstocks containing a heat-stable gene should be a priority in order to control RKN in tomatoes grown at high soil temperatures. This will allow earlier planting and prolong the production season.

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