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Orijinal araştırma (Original article)

Response of heat-stable tomato genotypes to *Mi-1* virulent root-knot nematode populations

Mi-1 virülent kök-ur nematod populasyonlarına yüksek sıcaklıkta dayanıklı domates genotiplerinin tepkisi

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Summary

Tomato is one of the most important vegetables cultivated worldwide. Many pests and pathogens cause significant yield decline in growing fields. Root-knot nematodes are known to be devastating pathogens on tomato. Resistant varieties carrying *Mi-1* gene have been effectively used to control of root-knot nematodes. However, the efficiency of *Mi-1* has been especially restricted by virulent root-knot nematode populations in cultivated tomatoes. Therefore, new resistant genetic varieties are required in growing fields. Heat-stable wild tomato species are known to be resistance to *Meloidogyne* populations at high soil temperature. To our knowledge, there are limited studies on response of heat-stable materials to different virulent root knot nematode populations under 28 ^{o}C. In the present study, reactions of heat stable materials to different virulent isolates of *M. incognita* and *M. javanica* were investigated at 24 ^{o}C soil temperature. Results showed that these materials did not confer resistance against *M. incognita* and *M. javanica* virulent isolates. Therefore, searching of new genetic sources resistant to virulent root-knot nematodes is required for breeding program.

Key words: Nematode, resistance, vegetable, virulence

Özet

Domates dünya çapında kültürü yapılan en önemli sebzelerden birisidir. Domates yetiştirilen alanlarda birçok zararlı patojen ve hastalık önemli ürün kayıplarına sebep olmaktadır. Kök-ur nematodları domatesin en önemli zararlısı olarak bilinmektedir. *Mi-1* geni taşıyan dayanıklı çeşitler kök-ur nematodlarını kontrol etmede etkin şekilde kullanılmaktadır. Bununla birlikte *Mi-1* geninin etkisi kültürü yapılan domateslerde, virülent kök-ur nematodları tarafından sınırlanmaktadır. Bu sebeple bu nematodlara dayanıklı yeni genetik çeşitlerin kullanılması gerekmektedir. Yüksek sıcaklığa dayanıklı yabani domates türlerinin yüksek sıcaklıkta *Meloidogyne* populasyonlarına dayanıklı olduğu bilinmektedir. Bu türlerin 28 ^{o}C'nin altındaki toprak sıcaklığında farklı virülent kök-ur nematod popülasyonlarına karşı tepkileri konusunda sınırlı sayıda çalışma bulunmaktadır. Bu çalışmada *M. incognita* ve *M. javanica*'nın farklı virülent izolatlarına yüksek sıcaklığa dayanıklı materyallerin tepkileri 24 ^{o}C toprak sıcaklığında testlenmiştir. Sonuçlar bu materyallerin *M. incognita* ve *M. javanica* virulent izolatlarına karşı dayanıklılık sağlamadığını göstermiştir. Bu sebeple ıslah programları için virülent kök-ur nematodlarına dayanıklı yeni genetik kaynakların araştırılması gerekmektedir.

Anahtar sözcükler: Nematod, dayanıklılık, sebze, virülenslik

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Introduction

Tomato is one of the most important vegetables grown in both open fields and protected cultivations in the world. Root-knot nematodes are one of the considerable pathogens causing significant yield losses in cultivated tomatoes worldwide. Host plant resistance is currently considered as the most efficient and environmentally safe method to control root-knot nematodes. Resistance to root-knot nematodes in tomato is conferred by the *Mi-1* gene. Currently, all commercial tomato varieties which are resistant to root-knot nematode carry the *Mi-1* gene. The occurrence of resistance breaking *Meloidogyne* populations could significantly reduce commercial utilization of *Mi-1* resistant tomato cultivars. The presence of *Mi-1* virulent root-knot nematode populations was reported in the vegetable growing fields in France, The United States of America and Turkey (Castagnone-Sereno, 1994; Kaloshian et al., 1996; Roberts et al., 1990; Devran & Söğüt, 2010). This situation creates a risk for tomato varieties bearing the *Mi-1* gene which is known to be overcome by virulent populations. Therefore, the incorporation of new resistant sources into cultivated tomatoes is required. The heat-stable materials, PI126443 and PI270435 were identified as resistant sources for some root-knot nematode species at high soil temperature 32 ^{o}C (Ammati et al., 1985). It was reported that a hybrid plant from cross between *S. peruvianum* PI270435-3MH and *S. peruvianum* PI 126443-1MH was susceptible to one *Mi*-virulent *M. incognita* isolate, but resistant to another *Mi*-virulent *M. incognita* isolate (Roberts et al., 1990). In another study, *S. peruvianum* PI 126443 accession was found resistant to *Mi-1* virulent *M. incognita* 557R isolate in soil temperature at 32 ^{o}C (Yaghoobi et al., 1995). Similarly, Veremis & Roberts (1996) reported that *S. peruvianum* PI 126443-1MH, PI 270435-3MH and, PI 270435-2R2 were resistant to *Mi*- virulent *M. incognita* 557R isolate at 25 ^{o}C and 32 ^{o}C. It was also reported that some clones of heat stable accessions PI126443 and PI 270435 were resistant to four geographically distinct *Mi-1* virulent root-knot nematode isolates at 25 ^{o}C soil temperature (Huang et al., 2004). If these accessions are found as being resistant to *Mi-1* virulent Turkish root knot nematode populations in soil temperature under 28 ^{o}C, they can be evaluate in tomato breeding programs. Then, they can be recommended for controlling nematode in tomato growing areas where virulent root-knot nematodes are present. To the best of our knowledge, since these materials have not been tested against *Mi-1* virulent Turkish root knot nematode, information about their performance is lacking.

The objective of the present study was to test ı) the heat stable materials PI126443 and PI270435 against different *Mi-1* virulent populations of *M. incognita* and *M. javanica* collected from geographical locations in the West Mediterranean region of Turkey at soil temperature of 24 ^{o}C ıı) compare response of *Mi-1* virulent root knot nematode isolates on these sources.

Materials and Methods

Plant material

Seeds of *S. lycopersicum* cultivars Tueza F_1 and Seval RN F_1, and *S. peruvianum* heat-stable materials PI 126443 and PI 270435 were kindly provided by Multi Tarım (Antalya, Turkey). The Tueza F_1 and Seval RN F_1 were used as susceptible and resistant controls, respectively, to compare response of root knot nematodes.

Nematode culture

Isolates of *M. incognita* and *M. javanica* were identified our previous studies by molecular methods (Devran & Söğüt, 2009). Their (a) virulent properties were also characterized with testing of resistant tomato plants carrying the *Mi-1* (Devran & Söğüt, 2010). Root-knot nematode isolates used in this study are given in Table 1. Avirulent isolates were used as control in these experiments.

Pure culture of root-knot nematode isolates was carried out according to previous study (Devran & Söğüt, 2010). Nematode isolates were maintained on susceptible fresh market tomato Tueza F_1. Egg masses were collected from infected roots using a small needle and hatched at room temperature. Second stage juveniles (J2) were counted under a light microscope. Plant materials were inoculated at the fourth true leaf stage with 1000 *M. incognita* and *M. javanica* juveniles 2. Plants were grown at 24 ^{o}C growth chamber under controlled conditions and harvested 8 weeks after inoculation. Root systems were carefully washed under tap water.

DNA isolation

Plant genomic DNA was extracted from young leaf tissue by using the Wizard Magnetic Kit (Promega) following the manufacturer's instructions. Nematode DNA was also isolated from five egg masses with DNAeasy Tissue and Blood Kit (Qiagen, Hilden, Germany) according to manufacturer' instructions.

PCR amplification

Pure culture isolates of *M. incognita* and *M. javanica* were confirmed by PCR analysis using the species-specific primers inc14F/inc14R (Randin et al., 2002) and Fjav/Rjav (Zijlstra et al., 2000). The presence of *Mi-1* gene in tested plants was checked by Mi23 marker (Seah et al., 2007).

The PCR reaction was performed in a total volume of 25 µL with 20 ng of DNA.The reaction mixture contained 2 mM MgCl2, 200 µM dNTPs, 0.4 µM of each primer, 2.5 µL 10XPCR buffer and 1 U Taq DNA polymerase. Amplification was performed in a thermal cycler DNA Engine PTC-200 (Bio-Rad, Hercules, CA) using the following conditions: 3 min at 94^{o}C, 35 cycles at 94 ^{o}C for 30 sn., 60 ºC (inc14F/inc14R, and Fjav/Rjav), 56 ºC (Mi23F/Mi23R) for 30 sn and 72^{o}C for 1 min with a final extension at 72 ^{o}C for 7 min. Amplified products were analyzed on a 2% agarose gel in 1X TAE buffer and visualized by ethidium bromide staining.

Growth chamber assay

Tomato seeds were germinated in viol containing steam-sterilized sandy soil, and 2-week old seedlings were transplanted singly to 250 ml plastic pots. Egg masses of root knot nematode were collected from the roots and counted according to nematode culture procedure mentioned above. Tomato plants with four true leaves were inoculated with 1000 Second stage juveniles each, and maintained at 24°C in a growth chamber. Five replications for each genotype were taken for the screening test in randomized block design. The plants were evaluated after 8 weeks of applying nematode inoculations. Inoculated plants were uprooted and roots were washed under tap water before scoring for root knot indices. Gall index and egg masses were assessed on a 0-5 scale according to Sasser et al. (1984) as follows: 0= no egg, 1 =1-2 egg masses, 2= 3-10 egg masses, 3:11-30 egg masses, 4:31-100 egg masses and 5: more than 100 egg masses per root system.

Data analysis

The egg mass number and the root gall index for each pot were subjected to analysis of variance (ANOVA) (SAS). The significance of the differences among plants 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 and Discussion

In order to control pure culture isolates of *M. javanica* and *M. incognita* were applied species-specific primers. *M. javanica* was identified by Fjav/Rjav primer produced 670 bp (Figure 1).

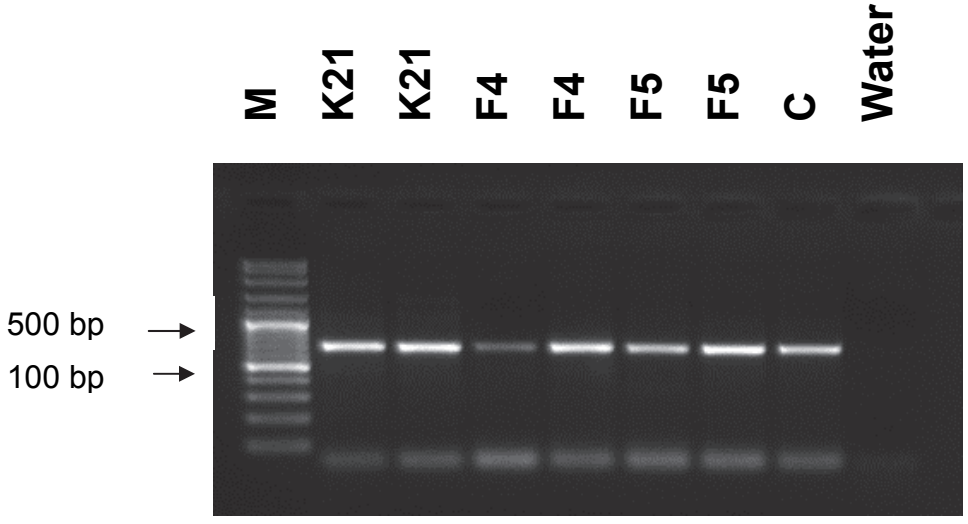

Figure 1. PCR profiles using species-specific primers of *Meloidogyne javanica*. M: Molecular weight marker, K21-F5: *M. javanica* isolates, C: Positive control.

Meloidogyne incognita was controlled using inc-K14-F/inc-K14-R produced approximately 400 bp (Figure 2).

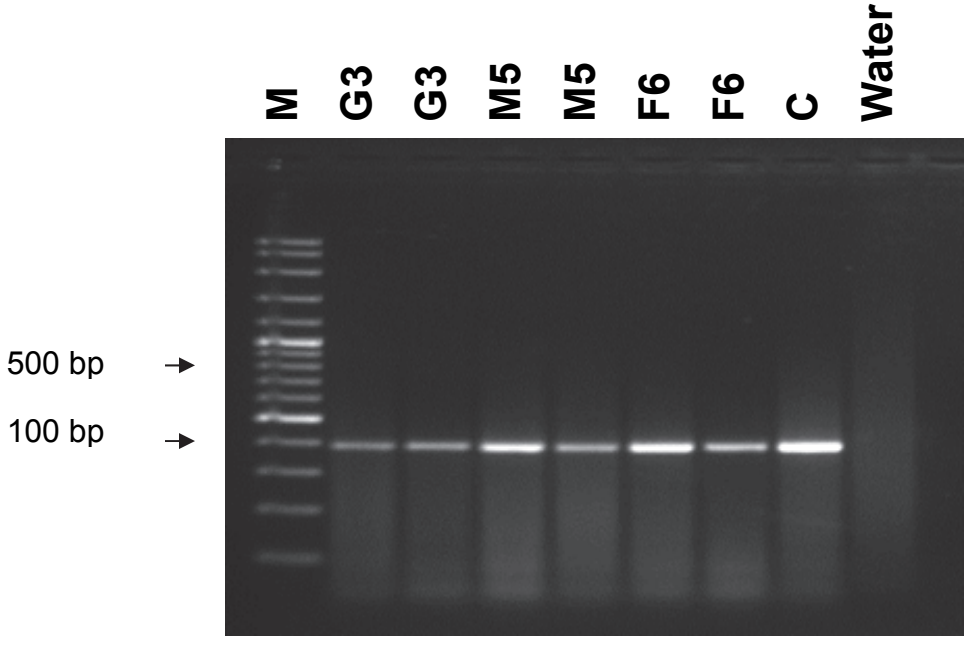

Figure 2. PCR profiles using species-specific primers of *Meloidogyne incognita*. M: Molecular weight marker, G3-F6: *M. incognita* isolates, C: Positive control.

Our finding showed accordance with previous studies (Zijlstra et al., 2000; Randig et al., 2002, Devran & Söğüt, 2009). Therefore, molecular results indicated that root-knot nematode species used in this study become pure culture.

As known, molecular markers linked to *Mi-1* gene have been developed for screening resistance to root-knot nematode in breeding program (Williamson et al., 1994; El Mehrach et al., 2005; Seah et al., 2007). The presence of the *Mi-1* gene in the plants was confirmed with Mi23 marker in the present study. PCR with Mi23 primer pairs yielded 380 bp and 430 bp fragments with homozygous resistant and susceptible plants, respectively. Heterozygous plants produced 380 bp and 430 bp fragments (Figure 3). Marker analysis showed that some of heat-stable plants were homozygous and heterozygous resistant, Tueza F_1 was susceptible and Seval F_1 were heterozygous resistant (Figure 3).

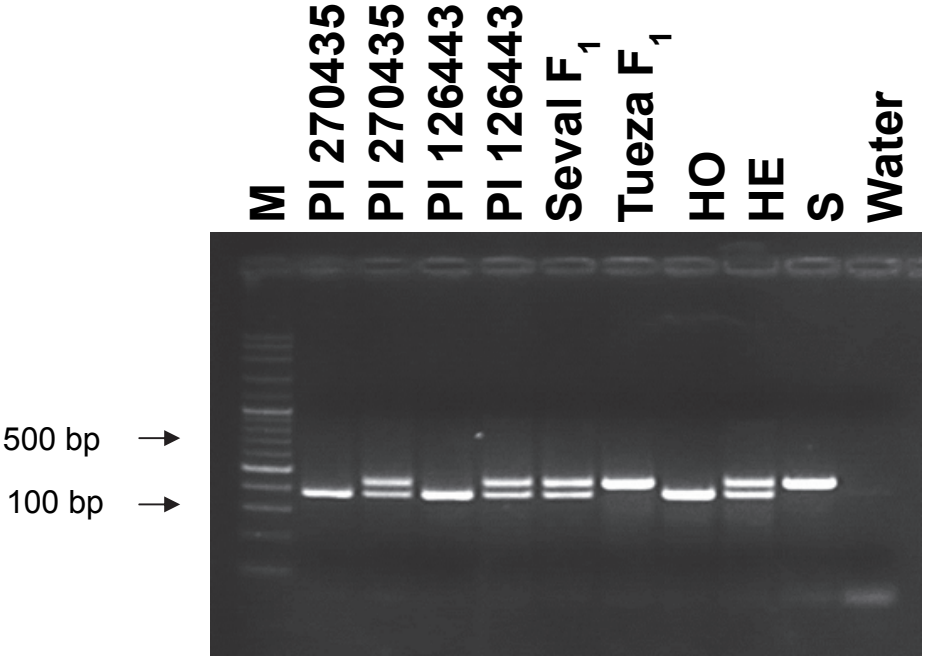

Figure 3. PCR profiles with Mi23 primer. M: Molecular weight marker (100 bp, Vivantis), HO: Homozygous resistant, HE: Heterozygous resistant, S: Susceptible.

Molecular and pathological results regarding Seval F_1 and Tueza F_1 were in accordance with declaration of breeding company. The Mi23 marker was reported a co-dominant marker linked to the *Mi-1* gene as being a valuable tool for the marker-assisted selection of root-knot nematode in tomato (Seah et al., 2007). Findings are also corresponded with study on comparison of molecular marker linked to *Mi-1* (Devran et al., 2013). Our results also showed this marker can be confidently used to screen heat stable sources bearing *Mi-1* gene for nematode resistance. It is known that *S. peruvianum* accessions are not self-pollinate (Rick, 1986; Taylor, 1986; Veremis et al., 1999). Therefore, these accessions showed both homozygous and heterozygous resistant properties according to molecular marker results. The heat-stable materials are not used practically in breeding programs due to incompatibility barriers with cultivated tomato (Taylor, 1986; Lefrancois et al., 1993). However, this incompatibility has been overcome by embryo rescue (Doğanlar et al., 1997). If these accessions were resistance to virulent root knot nematode, resistant gene(s) would be transferred into cultivated tomato via tissue culture methods such as embryo culture.

Avirulent and virulent of *M. incognita* and *M. javanica* isolates are used in this study (Table 1).

Table 1. Root-knot nematode isolates used in this study.

Code	Location	Species	Properties
K21	Finike	*M. javanica*	Avirulent
F4	Fethiye	*M. javanica*	Virulent
F5	Fethiye	*M. javanica*	Virulent
G3	Gazipaşa	*M. incognita*	Avirulent
M5	Gaziler	*M. incognita*	Virulent
F6	Fethiye	*M. incognita*	Virulent

Tomato cultivars (Tueza F_1 and Seval RN F_1) and *S. peruvianum* heat-stable materials (PI126443 and PI270435) were tested (a)virulent isolates of *M. incognita* and *M. javanica* at 24 ^oC soil temperature. Avirulent isolates of *M. incognita* and *M. javanica* showed similar reactions which were not significantly different on all plants tested (Table 2, 3).

Table 2. Egg masses and gall rating for avirulent K21 isolate of *M. javanica*

Plant	Number of egg masses	Gall rating (0-5)	Response	Mi23 Marker
PI 270435	0,0 ± 0,0 a	0,0 ± 0,0 a	R	RR
PI 126443	0,0 ± 0,0 a	0,0 ± 0,0 a	R	Rr
Seval F_1	1,2 ± 0,8 a	0,4 ± 0,3 b	R	Rr
Tueza F_1	167,4 ± 4,5 b	5,0 ± 0,0 c	S	rr

R: Resistant, S: Susceptible
Means in columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Table 3. Egg masses and gall rating for avirulent G3 isolate of *M. incognita*

Plant	Number of egg masses	Gall rating (0-5)	Response	Mi23 Marker
PI 270435	0,0 ± 0,0 a	0,0 ± 0,0 a	R	RR
PI 126443	2,4 ± 2,4 a	0,4 ± 0,4 a	R	RR
Seval F_1	1,8 ± 1,4 a	0,4 ± 0,3 a	R	Rr
Tueza F_1	157,0 ± 10,1 b	5,0 ± 0,0 b	S	rr

R: Resistant, S: Susceptible
Means in columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

The Tueza F_1, as expected, showed susceptible reaction to avirulent *M. incognita* and *M. javanica* isolates. It had the highest number of egg masses and gall on its roots. The number of egg masses and the root gall index scale were found more than 100 and 5, respectively (Table 2, 3). The Seval RN F_1, PI126443 and PI 270435 were resistant to avirulent isolates of *M. incognita* and *M. javanica*. The number of egg masses and the root gall index on their roots were very low or not detected (Table 2, 3). Therefore, three of the tested genotypes, Seval RN F_1, PI126443 and PI 270435, were evaluated as resistant to these isolates (Table 2, 3). However, there were no statistically considerable differences among resistant plants according to the number of egg mass and root gall index ($P \leq 0.05$). Devran et al. (2010) reported that PI126443 and PI 270435 were resistant to avirulent isolates of *M. incognita* at soil temperature 24 ^oC. In another study, results showed that tomato rootstocks carrying *Mi-1* gene were resistant isolates of root-knot nematodes (Cortada et al., 2009; Devran & Söğüt, 2010). Findings were in accordance with previous studies. As a result, since they carry on *Mi-1* gene, avirulent isolates did not produce both resistant cultivated plant and the heat stable materials as expected.

Virulent isolates of *M. incognita* and *M. javanica* showed similar reactions in plants tested (Table 4-7). There were significant differences between resistant plants (PI126443, PI 270435 and Seval F_1), and susceptible Tueza F_1 in the number of egg masses for virulent isolates of *M. javanica* (Table 4, 5). The *M. javanica* virulent isolates produced high ($P \leq 0.05$) egg masses on the susceptible tomato than resistant tomato variety and heat stable sources. However, the gall rating of virulent *M. javanica* populations was not statistically significant on all plants ($P \leq 0.05$) (Table 4, 5).

Table 4. Egg masses and gall rating for virulent F4 isolate of *M. javanica*

Plant	Number of egg masses	Gall rating (0-5)	Response	Mi23 Marker
PI 270435	145,6 ± 5,7 a	4,8 ± 0,2 a	S	RR
PI126443	148,0 ± 7,1 a	4,8 ± 0,2 a	S	RR
Seval F_1	131,0 ± 5,8 a	4,8 ± 0,2 a	S	Rr
Tueza F_1	167,6 ± 5,1 b	4,9 ± 0,1 a	S	rr

R: Resistant, S: Susceptible
Means in columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Table 5. Egg masses and gall rating for virulent F5 isolate of *M. javanica*

Plant	Number of egg masses	Gall rating (0-5)	Response	Mi23 Marker
PI 270435	145,2 ± 7,3 a	4,8 ± 0,2 a	S	RR
PI126443	148,0 ± 9,7 a	4,8 ± 0,2 a	S	RR
Seval F_1	145,2 ± 7,6 a	4,8 ± 0,2 a	S	Rr
Tueza F_1	173,0 ± 5,5 b	4,9 ± 0,1 a	S	rr

R: Resistant, S: Susceptible
Means in columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

The number of egg mass and gall rating of *M. incognita* virulent isolates was not statistically significant on all tested plants ($P \leq 0.05$) (Table 6, 7).

Table 6. Egg masses and gall rating for virulent M5 isolate of *M. incognita*

Plant	Number of egg masses	Gall rating (0-5)	Response	Mi23 Marker
PI 270435	153,4 ± 10,0 a	4,8 ± 0,2 a	S	RR
PI 126443	158,8 ± 9,5 a	4,8 ± 0,2 a	S	RR
Seval F_1	147,8 ± 8,6 a	4,8 ± 0,2 a	S	Rr
Tueza F_1	153,6 ± 9,2 a	5,0 ± 0,0 a	S	rr

R: Resistant, S: Susceptible
Means in columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

Table 7. Egg masses and gall rating for virulent F6 isolate of *M. incognita*

Plant	Number of egg masses	Gall rating (0-5)	Response	Mi23 Marker
PI 270435	139,6 ± 12,1 a	5,0 ± 0,0 a	S	RR
PI 126443	129,2 ± 8,7 a	4,8 ± 0,2 a	S	RR
Seval F_1	144,6 ± 13,8 a	5,0 ± 0,0 a	S	Rr
Tueza F_1	151,0 ± 8,8 a	5,0 ± 0,0 a	S	rr

R: Resistant, S: Susceptible
Means in columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Occurrence of virulent root-knot nematode populations has been reported in different regions of the world (Tzortzakakis & Gowen, 1996; Roberts & Thomason, 1989; Ornat et al., 2001; Tzortzakakis et al., 2005). In Turkey, a preliminary study was carried out on presence of virulent root knot nematode population in the West Mediterranean region, and results indicated the virulent *M. incognita* and *M. javanica* populations were found 11.7% and 21.4% depending on sampling fields, respectively (Devran & Söğüt, 2010). Since the occurrence of resistance breaking *Meloidogyne* populations could significantly reduce commercial utilization of *Mi-1* resistant tomato cultivars, new resistant sources are required for controlling of *Mi-1* virulent root-knot nematode populations. The heat-stable materials, PI126443 and PI270435 can be used as resistant sources for virulent root-knot nematode species. Huang et al. (2004) compared the reproduction of four *Mi*-virulent *M. incognita* isolates on heat stable sources *S. peruvianum* PI 270435-2R2, PI126443-1MH and PI 270435-3MH at 22 ^{o}C and 25 ^{o}C and identified as resistant these sources. Similarly, Veremis & Roberts (1996) reported that *S. peruvianum* PI 126443-1MH, PI 270435-3MH and, PI 270435-2R2 were resistant to *Mi*- virulent *M. incognita* 557R isolate at 25 ^{o}C and 32 ^{o}C. However, It was reported that a hybrid plant from cross between *S. peruvianum* PI270435-3MH and *S. peruvianum* PI 126443-1MH was susceptible to one *Mi*-virulent *M. incognita* isolate, but resistant to another *Mi*-virulent *M. incognita* isolate (Roberts et al., 1990). This study showed that differences in gall rating between heat-stable materials and resistant variety were not significant. Findings showed virulent isolates reproduced in the plants tested successfully. These results indicated that heat stable sources can show different reaction properties against virulent root knot nematode populations. Findings show that the gene(s) which is resistant to virulent root knot nematode population has not wide spectrum resistance properties in terms of their populations. Therefore, usage of the gene(s) is restricted worldwide. Similarly, genes providing species specific resistance to some root knot nematodes have been reported in *S. huaylasense* accession LA 1358 (Cortada et al., 2010).

In conclusion, resistant varieties are one of the most effective management strategies for control root knot nematode. The *Mi-1* gene which is resistant to root knot nematodes is widely used to control *Meloidogyne* spp. in tomato breeding programs. Since tomatoes bearing *Mi-1* gene breaks down irreversibly at soil temperatures above 28 ^{o}C (Dropkin, 1969), it is important that planning of planting in tomato growing areas due to soil temperature. Hence, this approach can give opportunity for effective of the *Mi-1* gene in growing fields. However, proliferation of virulent populations is restricted usage of varieties carrying the *Mi-1* gene in the tomato production. Therefore, searching of new sources with resistant to virulent root knot nematode can be studied as a priority strategy in the future researches.

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