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Original article (Orijinal araştırma)

Response of tomato plants carrying *Mi-1* gene to *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 under high soil temperatures[1]

Yüksek toprak sıcaklıklarında *Mi-1* genini taşıyan domates bitkilerinin *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949'a tepkisi

Tevfik ÖZALP[2] **Zübeyir DEVRAN[2*]**

Abstract

The *Mi-1* gene conferring resistance to root-knot nematodes in tomato breaks down at soil temperatures above 28ºC. To understand this phenomenon, the reactions of susceptible and resistant tomatoes to *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 were separately investigated under four soil temperatures, 25, 28, 30 and 32ºC, and at six time periods, 6, 12, 24, 48, 120 and 168 h. The study was conducted between 2015 and 2016 in growth chambers. In the first experiment, the plants were separately exposed to soil temperatures for the same six periods before nematode inoculation and then transferred to a growth chamber with 25ºC. Reproduction factor (Rf) for nematode on resistant plants was <1, while the Rf for susceptible plants was >1. Results indicated that the resistance provided by *Mi-1* persisted under all soil temperatures. In the second experiment, the seedlings were simultaneously inoculated with *M. incognita* when soil temperatures reached 25, 28, 30 and 32ºC, and held in soil temperatures for the same six periods, then transferred to a growth chamber with 25ºC soil temperature. Rf in heterozygous resistant plants exposed to 32ºC soil temperature for ≥48 h was >1. This study indicated that the resistance in plants held at 32ºC soil temperature for ≥48 h lost its effect.

Keywords: Duration, *Mi-1* gene, resistance, root-knot nematodes, soil temperature, tomato

Öz

Domateste kök ur nematodlarına karşı dayanıklılık sağlayan *Mi-1* geni 28ºC'nin üzerindeki toprak sıcaklılarında kırılmaktadır. Bu durumu anlamak için *Meloidogyne inco*gnita (Kofoid & White, 1919) Chitwood, 1949' ya hassas ve dayanıklı bitkiler, dört toprak sıcaklığında (25, 28, 30 ve 32ºC) ve 6 sürede (6, 12, 24, 48, 120 ve 168 saat) ayrı ayrı incelenmiştir. Bu çalışma 2015 ve 2016 yılları arasında iklim odalarında yürütülmüştür. İlk denemede bitkiler nematod inokulasyonundan önce aynı altı zaman periyodu için 25, 28, 30 ve 32ºC toprak sıcaklıklarına maruz bırakılmış, daha sonra 25ºC'deki iklim odasına aktarılmıştır. Nematodların üreme faktörü (Rf) dayanıklı bitkilerde 1'den büyük hassas bitkilerde ise 1'den küçük bulunmuştur. İlk denemenin sonucu, *Mi-1* tarafından sağlanan dayanıklılığın belirtilen toprak sıcaklıklarında kırılmadığını göstermiştir. İkinci denemede toprak sıcaklıkları 25ºC, 28ºC, 30ºC ve 32ºC'ye ulaştığında eş zamanlı olarak *M. incognita* inokulasyonu yapılmış ve adı geçen sürelerde toprak sıcaklığına maruz bırakılmıştır. Daha sonra bitkiler 25ºC toprak sıcaklığına sahip iklim odasına aktarılmıştır. 32ºC toprak sıcaklığına, 48 saat ve üzerinde maruz bırakılan heterozigot dayanıklı bitkilerde Rf değeri >1 olarak bulunmuştur. Bu sonuçlar 32ºC toprak sıcaklığına, 48 saat ve üzeri maruz bırakılan bitkilerdeki dayanıklılığın etkisini yitirdiğini göstermiştir.

Anahtar sözcükler: Süre, *Mi-1* geni, dayanıklılık, kök-ur nematodları, toprak sıcaklığı, domates

[1] This study represents first author's master thesis.

[2] Akdeniz University, Agricultural Faculty, Plant Protection Department, 07058, Antalya, Turkey
* Corresponding author (Sorumlu yazar) e-mail: zdevran@akdeniz.edu.tr
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Introduction

Tomato is a widely grown vegetable with an annual worldwide yield of about 173 million ton/year (FAO, 2014). It is also a major dietary source of lycopene, which reduces the risk of developing heart disease and cancer (Clinton et al., 1996; Arab & Steck, 2000). As with other crop plants, many pests and pathogens attack cultivated tomatoes, damaging both quality and quantity of production. Root-knot nematodes (RKN), *Meloidogyne* spp., are considered a major pest in tomato-growing areas. They feed and develop on plant roots, resulting in the formation of galls or knots, which cause reduced water and nutrients uptake (Duncan & Noling, 1998). As a result, plants may exhibit unspecific symptoms, which can be confused with water and nutrient deficiency (Duncan & Noling, 1998). In addition, plants can become more susceptible to fungal and bacterial diseases (Taylor & Sasser, 1978; Siddiqui et al., 2014; Al-Hazmi & Al-Nadary, 2015; Lobna et al., 2016).

Management of RKN is difficult due to their polyphagous nature (Siddiqi, 2000), reproduction capacity (Moens et al., 2009), being soilborne pathogens (Starr et al., 1989; Manzanilla-López & Starr, 2009) having a wide range of host (Hussey, 1985). Pesticides, resistant cultivars and rootstocks are commonly used to manage RKN (Devran et al., 2010). Nematicides have been widely used to RKN control. However, the use of some nematicides has been restricted owing to human health and environmental concerns (Devran et al., 2008; Moens et al., 2009; Devran et al., 2013). Therefore, growing resistant cultivars is considered an alternative and environmentally-friendly strategy to manage RKN (Devran et al., 2013). In tomatoes, *Mi-1* controls resistance to RKN. This gene was introgressed into cultivated tomato from wild tomato *Solanum peruvianum* L. (Solanaceae) in the 1940s (Smith, 1944). It confers resistance against three species of RKN: *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, *Meloidogyne javanica* (Treub, 1885) and *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Roberts & Thomason, 1986). *Mi-1* gene-mediated resistance is characterized by a localized hypersensitive response to the attempt of a nematode to initiate a feeding site in root cells (Dropkin, 1969a). This gene has been successfully incorporated into many commercially available tomato cultivars and is currently the only source of RKN resistance in commercial tomatoes (Devran et al., 2010; Seid et al., 2015). This gene also confers resistance to the aphid *Macrosiphum euphorbiae* (Thomas, 1878) (Homoptera: Aphididae) and *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) biotypes Q and B (Rossi et al., 1998; Nombela et al., 2003). However, the resistance conferred by *Mi-1* gene has limited. The gene loses its effectiveness at soil temperatures of above 28ºC (Dropkin, 1969b). However, there are discrepancies in information about the recovery and the duration of resistance breakdown provided by *Mi-1* gene at high soil temperatures. Dropkin (1969b) reported that the phenotypic expression of resistance provided by the gene changed at temperatures above 28ºC. Araujo et al. (1982a) observed numerous egg masses on roots of plants incubated for 3 d at 25ºC, then moved to 32.5ºC for 27 d in their reciprocal experiment. In contrast, Haroon et al. (1993) reported that there were no galls on in vitro root explant tissues of resistant tomatoes maintained at 28ºC or 30ºC for 10 d; however, galls were present on roots at 33ºC and increased at 37 and 40ºC. Similarly, when in vitro root explants without *Mi-1* gene and resistant genotypes carrying *Mi-1* gene were inoculated with *M. incognita* and *M. arenaria* at 28, 31, 34 and 37ºC, heterozygous and homozygous genotypes were equally resistant to both RKN species and genotypes lacking *Mi-1* gene were susceptible to all temperatures. In addition, the resistance level was maintained fully at 31ºC, maintained partially at 34ºC and lost at 37ºC (Abdul-Baki et al., 1996). In several studies, tomato plants with the *Mi-1* gene were inoculated with *M. incognita* after heat treatment at five temperatures between 27 and 38ºC. Consequently, the seedlings showed increased susceptibility to *M. incognita* above 30ºC with the maximum reached at 34ºC (Zacheo et al., 1995). Carvalho et al. (2015) reported that for plants exposed to ambient temperatures of 35ºC for 3 h daily (midday), resistance level could be recovered by maintaining them in a low temperature for 6 d. Unlike under controlled conditions, when the soil temperature reached above 28ºC under greenhouse, *Mi-1*-mediated resistance reduced greatly (Cortada et al., 2008).

Although the effect of temperatures on RKN resistance under different temperatures has been extensively studied, the results remain inconclusive. Unlike many previous studies, in this study, the soil temperature was constantly monitored with a probe, and the inoculation time and heat exposure periods recorded. This study examined the reactions of (a) tomatoes bearing *Mi-1* gene to *M. incognita* at high soil temperatures, and (b) tomatoes cultivars with or lacking the *Mi-1* gene to *M. incognita* exposed to soil temperatures of 25, 28, 30 and 32ºC for 6, 12, 24, 48, 120 and 168 h. The purpose was to determine the time of resistance break down in heterozygous and homozygous tomato cultivars at the *Mi* locus exposed to high soil temperatures and then simultaneously inoculated with *M. incognita*.

Material and Methods

The study was conducted at Plant Protection Department, Faculty of Agriculture Akdeniz University, Antalya, Turkey between 2015 and 2016.

Plant material

The homozygous tomato cv. Tueza F1 without the *Mi-1* gene (*mimi*), the heterozygous cv. Seval F1 at the *Mi-1* locus (*Mimi*) and the homozygous cv. Browny F1 at the *Mi-1* locus (*MiMi*) were used in the bioassay. Tomato seedlings were provided by Multi Tohum Tar. San Tic. A.Ş. (Antalya, Turkey). Individual tomato seedlings were transferred to 250-mL pots including sterilized sandy soil.

Nematode isolate

The S6 isolate of *M. incognita* race 2 was used in the experiments. This isolate was previously described and characterized (Devran & Söğüt, 2009, 2011). The isolate was maintained in the susceptible tomato cv. Tueza F1. Tomato plants were inoculated with 1000 J2 (Devran et al., 2010; Devran & Söğüt, 2014; Mıstanoğlu et al., 2016) and maintained in a growth chamber at 25±0.5ºC with 16:8 h L:D photoperiod and 65% RH. Eight weeks after nematode inoculation the tomato plants were uprooted and the roots were washed free of soil. Then, the egg masses were handpicked and incubated in a petri dish at room temperate. The juveniles (J2) were collected at first 24 h, counted and inoculated in new tomato plants in the same day or stored in refrigerator at 4ºC for 2 d, until inoculation.

DNA isolation

Plant genomic DNA and nematode DNA were isolated according to previous studies (Devran & Söğüt, 2009; Devran et al., 2013).

PCR amplification

The S6 isolate of *M. incognita* race 2 was verified the use of Inc14F/Inc14R primers (Randig, 2002). DNA of *M. arenaria* (K18) and *M. javanica* (AKS2) isolates was included as controls. The K18 and AKS2 isolates were identified and characterized in previous studies (Devran & Söğüt, 2009, 2011). The presence of *Mi-1* gene in tomato plants was verified using the *Mi23* marker (Seah et al., 2007). All PCR reactions were performed according to previous studies (Devran & Söğüt, 2009, 2014; Devran et al., 2013).

Effect of high temperatures on tomato resistance against *M. incognita*

Two different experimental designs were used, a) plants heat treated before nematode inoculation, and b) plants heat treated and inoculated simultaneously. Soil temperature in pots was continuously monitored with a probe and registered.

Experiment 1: Tomato seedlings (five replicates/treatment) with four true leaves individually exposed to soil temperatures of 25, 28, 30 and 32ºC for 6, 12, 24, 48, 120 and 168 h (Figure 1a). Then, the plants were transferred to a growth chamber at 25ºC with 16:8 h L:D photoperiod and 65% RH. When the soil temperature reached 25ºC, tomato seedlings were inoculated with 1000 freshly hatched *M. incognita* J2s (Figure 1b). The experiment was set up, performed and then repeated.

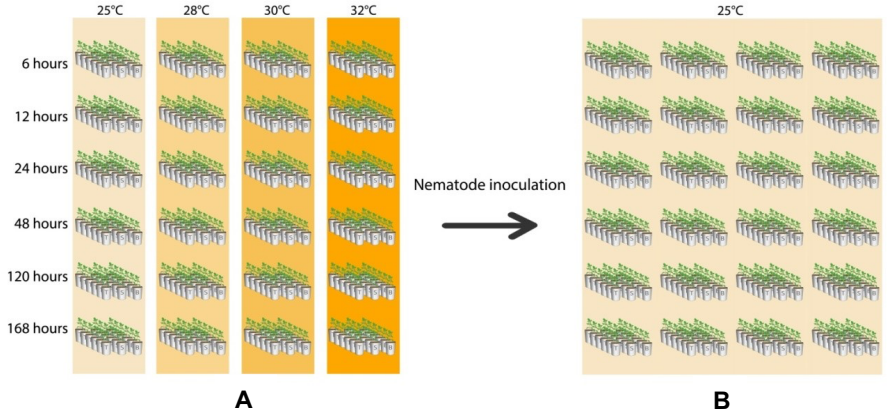

Figure 1. Design of the experiments to study the effect of high temperatures on tomato resistance. A) Tomato seedlings were individually exposed to four soil temperatures for different time periods, before inoculation with *Meloidogyne incognita* second-stage juveniles (J2); B) Tomato seedlings were exposed to different soil temperatures, inoculated with J2 and maintained for different time periods at 25 ºC.

Experiment 2: The tomato plants (five replicates/treatment) were exposed to soil temperatures of 25, 28, 30 and 32ºC for 6, 12, 24, 48, 120 and 168 h. When soil reached the designated temperature, plants were inoculated with 1000 freshly hatched (<24 h) *M. incognita* J2s (Figure 2a). The plants were held in soil temperatures for the same six periods and then transferred to a growth chamber at 25ºC, as referred before, until the end of the experiment (Figure 2b). The experiment was designed, conducted and then repeated.

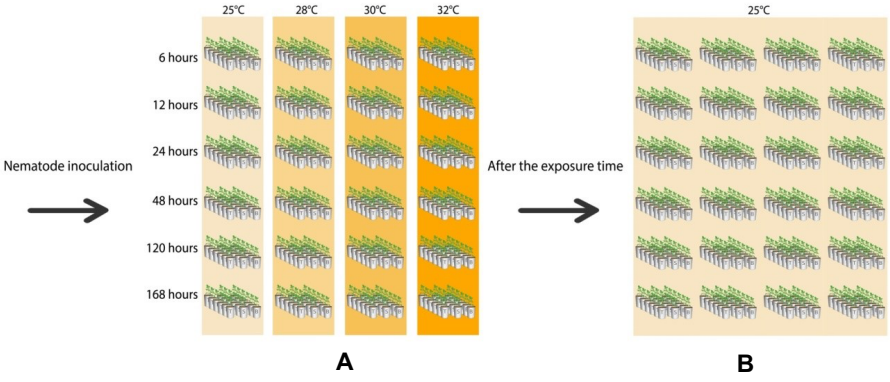

Figure 2. Design of the experiments to study the effect of high temperatures on tomato resistance A) The tomato seedlings separately exposed to four soil temperatures for different time periods and inoculated with *Meloidogyne incognita* second-stage juveniles; B) Plants transferred to a growth chamber with 25ºC.

Sixty day after inoculation the plants were evaluated. The number of galls and egg masses per root system was recorded in all experiments. Galls and egg masses were counted under a stereomicroscope. J2 were recovered from the soil of each pot (100 g soil/pot) using a modified Baermann funnel technique within 2 d (Hooper, 1986). Reproduction factor (RF; i.e., final J2 population density/initial nematode population, 1000 J2s) was calculated (Ferris, 1985). The data (number of egg masses, galls and J2s) data were log transformed [log10(x+1)] and analyzed by ANOVA. The significant differences within treatments were tested using LSD. The statistical analysis was performed according to SAS program (v. 9.0 for Windows; SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Confirmation of nematode isolate identification and detection of *Mi-1* gene

The *M. incognita* isolate S6 identification was confirmed by PCR using species-specific primers (Inc14F/Inc14R), which produced an amplicon of about 400 bp, as expected (Figure 3a).

The *Mi-1* gene was verified using the *Mi23* marker. This marker allows the amplification of 430-bp and 380-bp fragments for tomato cultivars without the *Mi-1* gene (*mimi*) and homozygous tomato cultivars at the *Mi* locus (*MiMi*), respectively. Heterozygous tomato cultivars (*Mimi*) presented the two fragments, as expected (Figure 3b).

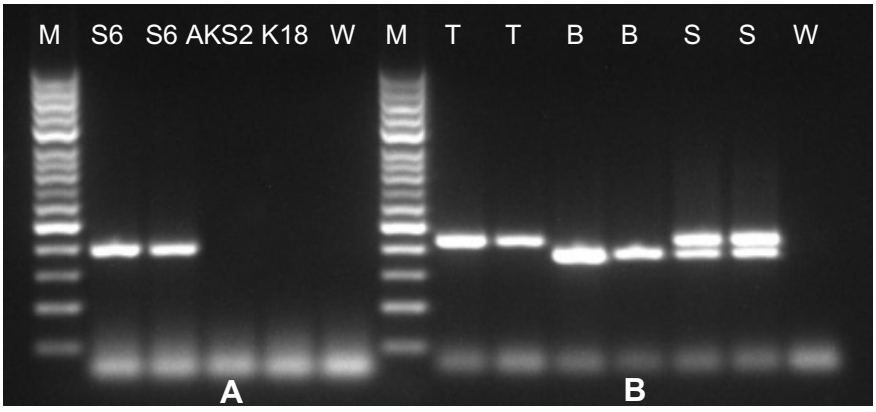

Figure 3. A) Amplified DNA of *Meloidogyne incognita* using primers Inc14F/Inc14R and B) PCR products of tomato cultivars using primers Mi23F/R. M: Marker (100 bp DNA Ladder, GeneAll, Seoul, Korea), S6: *M. incognita*, AKS2: *M. javanica*, K18: *M. arenaria*, T: Tueza F1 (*mimi*), B: Browny F1 (*MiMi*), S: Seval F1 (*Mimi*), W: Negative control.

Effect of high temperatures on tomato resistance

Experiment 1: At the end of the experiment, the number of J2s, egg masses and galls was evaluated for all plants (Table 1). The data indicated the resistance conferred by *Mi-1* persisted at the soil temperatures tested. Rf values of *M. incognita* on tomato plants (*mimi*) was >1 while on tomato cultivars (*MiMi* or *Mimi*) were <1, as expected (Table 1). Rf value of plants exposed to heat treatment at 32ºC is shown in Table 1.

Experiment 2: The tomato seedlings exposed to soil temperatures for the six time periods at the time of inoculation and parameters were evaluated (Table 2). The data showed marginal increases in the number of galls and egg masses on the roots of tomato plants (*MiMi*) held at 30ºC soil temperature for >120 h (Table 2). However, resistance did not break down and Rf values of *M. incognita* on homozygous (*MiMi*) and heterozygous (*Mimi*) tomato plants were <1 (Table 2). The number of egg masses on the roots of cv. Browny F1 (*MiMi*) held at 32ºC did not change at 6 and 12 h but increased to statistical significance at 24 h and markedly increased >48 h. The Rf of *M. incognita* on tomato seedlings (*Mimi*) at 32ºC for ≥48 h was >1. However, the results also demonstrated that the Rf of nematodes on tomato plants (*MiMi*) exposed to 32ºC soil temperature for 120 and 168 h were >1 (Table 2). So, the present study demonstrates that *Mi-1* gene-mediated resistance in tomato breaks down in plants held at 32ºC for ≥48 h.

Table 1. Number of galls, egg masses and Rf of *Meloidogyne incognita* on tomato cultivars exposed to different temperatures before inoculation with 1000 second-stage juveniles

Soil temperature	Parameters	Tomato cultivars*	Hours of exposure					
			6	12	24	48	120	168
25ºC	Gall	Tueza F1	148.4 a**	127.8 a	158.0 a	136.6 a	168.2 a	95.0 a
		Seval F1	4.8 b	0.4 e	0.8 de	2.6 bcd	2.0 bcde	3.4 bc
		Browny F1	0.4 e	3.6 b	2.0 cde	1.6 bcde	1.6 cde	2.0 cde
	Egg Mass	Tueza F1	83.0 a	44.4 b	65.2 a	70.4 a	77.8 a	61.8 a
		Seval F1	0.6 c	0.0 d	0.2 cd	0.4 cd	0.0 d	0.2 cd
		Browny F1	0.0 d	0.2 cd	0.2 cd	0.0 d	0.0 d	0.0 d
28ºC	Gall	Tueza F1	122.4 a	127.0 a	87.4 b	111.2 a	81.0 ab	89.6 ab
		Seval F1	1.2 de	4.8 c	3.4 cd	0.0 e	1.4 de	2.2 de
		Browny F1	0.2 e	0.4 e	0.0 e	0.6 e	0.0 e	0.0 e
	Egg Mass	Tueza F1	76.0 a	65.8 ab	50.0 c	58.6 ab	40.2 bc	40.8 abc
		Seval F1	0.2 de	0.6 de	0.4 de	1.6 d	0.0 e	0.2 de
		Browny F1	0.2 de	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e
30ºC	Gall	Tueza F1	149.2 a	89.2 ab	106.4 ab	86.0 ab	66.2 b	124 a
		Seval F1	2.0 de	1.8 de	5.6 c	4.0 cd	1.8 de	1.4 de
		Browny F1	0.2 e	1.2 de	1.4 e	0.6 e	1.2 e	1.0 e
	Egg Mass	Tueza F1	46.6 a	58 a	52.4 a	48.8 a	14.6 b	74.4 a
		Seval F1	0.2 c	0.4 c	0.2 c	0.4 c	0.4 c	0.8 c
		Browny F1	0.2 c	0.6 c	0.4 c	0.0 c	0.0 c	0.4 c
32ºC	Gall	Tueza F1	81.8 a	124.8 a	98.6 a	126.2 a	125.4 a	79.5 a
		Seval F1	0.6 de	4.6 b	4.4 b	3.2 bc	0.0 e	1.2 cde
		Browny F1	1.2 cd	0.4 de	0.6 de	1.2 cd	0.4 de	0.0 e
	Egg Mass	Tueza F1	42.8 a	48.2 a	50.4 a	54.2 a	57.4 a	54.5 a
		Seval F1	0.0 b	0.0 b	0.0 b	0.2 b	0.4 b	0.0 b
		Browny F1	0.4 b	0.0 b	0.0 b	1.0 b	0.2 b	0.0 b
	Rf	Tueza F1	2.63 c	3.90 b	4.12 b	1.12 d	5.08 b	7.91 a
		Seval F1	0.004 e	0.012 e	0.0 e	0.0 e	0.04 e	0.004 e
		Browny F1	0.0 e	0.004 e	0.008 e	0.008 e	0.0 e	0.0 e

* Tueza F_1: without *Mi* gene (*mimi*), Seval F_1: heterozygous (*Mimi*), Browny F_1: homozygous carrying the Mi gene (*MiMi*).

** Means with in the temperature sharing the same letter are not significantly different from each other at $P = 0.05$ according to the LSD. Untransformed data shown, statistical analysis was performed on log(x+1) transformed data.

Table 2. Number of galls, egg masses and Rf of *Meloidogyne incognita* on tomato cultivars exposed to different temperatures after inoculation with 1000 second-stage juveniles

Soil temperature	Parameters	Tomato cultivars*	Hours of exposure					
			6	12	24	48	120	168
25ºC**	Gall	Tueza F1	-	-	-	-	-	168.2 a
		Seval F1	-	-	-	-	-	2.0 b
		Browny F1	-	-	-	-	-	0.0 c
	Egg Mass	Tueza F1	-	-	-	-	-	84.4 a
		Seval F1	-	-	-	-	-	0.0 b
		Browny F1	-	-	-	-	-	0.0 b
28ºC	Gall	Tueza F1	81.0 a***	66.2 a	71.0 a	75.5 a	69.5 a	63.2 a
		Seval F1	3.2 bcd	1.0 ef	3.0 bcd	4.5 bc	2.5 cde	5.0 b
		Browny F1	0.0 f	1.5 cde	0.7 ef	1.0 ef	2.0 cde	1.2 de
	Egg Mass	Tueza F1	54.2 a	47.5 a	62.7 a	57.5 a	51.5 a	45.5 a
		Seval F1	0.7 b	0.5 bc	0.0 c	1.0 bc	0.0 c	0.5 bc
		Browny F1	0.0 c	0.2 bc	0.0 c	0.2 bc	0.0 c	0.5 bc
30ºC	Gall	Tueza F1	158.6 a	138.6 a	153.2 a	157.0 a	138.2 a	126.0 a
		Seval F1	3.0 bcde	3.6 bcd	1.4 defg	1.2 efgh	4.6 bc	4.0 bc
		Browny F1	1.0 fgh	0.4 gh	0.6 gh	0.2 h	5.2 b	2.0 cdef
	Egg Mass	Tueza F1	89.2 a	77.4 a	74.4 a	78.4 a	78.6 a	58.2 a
		Seval F1	0.6 de	0.2 e	0.2 e	0.2 e	1.6 cd	2.8 c
		Browny F1	0.0 e	0.0 e	0.0 e	0.0 e	4.8 b	0.6 de
32ºC	Gall	Tueza F1	108.2 a	129.8 a	131.2 a	132.8 a	94 ab	101.2 a
		Seval F1	0.8 hı	1.0 hı	4.0 g	17.4 f	56.2 bc	37.6 cd
		Browny F1	1.4 hı	0.8 ı	2.0 h	18.0 ef	39.4 dc	26.6 de
	Egg Mass	Tueza F1	71.2 a	109.4 a	73.6 ab	88.4 a	35.8 d	63.4 abc
		Seval F1	0.4 h	0.4 h	2.4 g	16.2 f	33.2 de	41.4 bcd
		Browny F1	0.2 h	0.2 h	1.2 h	19.8 ef	39.6 cd	22.2 def
	Rf	Tueza F1	12.75 abc	27.49 a	15.51 ab	9.27 bcd	5.64 cde	7.59 bcd
		Seval F1	0.07 ij	0.39 hij	0.33 hij	2.78 efg	3.86 def	4.72 def
		Browny F1	0.004 j	0.01 ij	0.07 ij	0.86 ghij	1.52 fgh	1.05 ghi

* Tueza F_1: without *Mi* gene (*mimi*), Seval F_1: heterozygous (*Mimi),* Browny F_1: homozygous carrying the Mi gene (*MiMi).*
** The experiment was continuously conducted at 25 ºC. Only one data was given in a column (168 hours).
*** Means with in the temperature sharing the same letter are not significantly different from each other at $P = 0.05$ according to the LSD. Untransformed data shown, statistical analysis was performed on log(x+1) transformed data.

It has been shown that some accessions of *S. peruvianum* are resistant to some RKNs at high soil temperature (Ammati et al., 1986). However, these genes are not commercially available for tomato cultivation. Therefore, *Mi-1* gene is the only commercially available resistance gene against some RKN and is widely used to RKN management in tomato-growing areas (Devran et al., 2010; Seid et al., 2015). However, this gene breaks down at high soil temperatures. Therefore, the effective utilization of the gene is restricted in tomato-growing areas.

Validation of *M. incognita* isolate and presence/absence of *Mi-1* gene on tomato cultivars is required before experiments. In the present study, this validation was performed using specific primers and our findings were in accordance with earlier studies (Randig et al., 2002; Devran & Söğüt, 2009, 2014; Devran et al., 2013).

Several studies have been conducted on the breakdown of *Mi-1* gene at high soil temperatures; however, results indicate a discrepancy in information about the duration of resistance breakdown at high soil temperatures. In this study, two experiments were conducted to clarify this phenomenon. In the first experiment, when plants were exposed to soil temperatures for different time periods and inoculated with *M. incognita* J2 at 25ºC, the resistance provided by *Mi-1* gene did not break down. Therefore, the resistance did not break down in plants exposed to high soil temperatures before nematode inoculation. Conversely, in other previous study, seedlings were exposed to high soil temperatures before nematode inoculation and then infected with *M. incognita*. Results indicated that the seedlings became increasingly susceptible to *M. incognita* above 30ºC and were completely susceptible at 34ºC (Zacheo et al., 1995). The difference may be due to *M. incognita* inoculum level. Araujo et al. (1982b) reported that temperature and inoculum level produced quantitative differences in resistance for both species of *Meloidogyne* with 28 d of incubation. In another study, Carvalho et al. (2015) reported that for plants exposed to 35ºC for 3 h daily (midday), resistance level could be recovered by maintaining them in a low temperature for 6 d. However, other studies reported that resistance could not be recovered (Araujo et al., 1982a; Abdul-Baki et al., 1996). This study revealed that resistance did not break down in plants exposed to high soil temperatures before nematode inoculation. This study showed that the resistance broke down in plants held at 32ºC soil temperature for ≥48 h, which is consistent with Dropkin (1969b). Although susceptible and resistant seedlings were inoculated with 20, 100 and 200 *M. incognita* J2 at 32.5ºC soil temperature, there were fewer egg masses on resistant plant roots than on susceptible plant roots. However, when plants were inoculated with 1000 and 2000 *M. incognita* J2, both plants showed comparable number of egg masses (Araujo et al., 1982b). Our results showed that egg masses in resistant tomato seedlings (*MiMi*) inoculated with 1000 *M. incognita* J2 were fewer than in susceptible plants (*mimi*). In another study, Abdul-Baki et al. (1996) reported that the resistance level in in vitro root explants was fully maintained at 31ºC, partially maintained at 34ºC and lost at 37ºC.

Some studies have also been conducted on the effect of temperatures on plants carrying *Mi-1* gene under greenhouse conditions. Cortada et al. (2008) reported that resistance conferred by *Mi-1* gene reduced greatly in soil temperature above 28ºC under greenhouse conditions in summer. The results showed differences on performance of plants carrying *Mi-1* gene under greenhouse conditions.

In conclusion, the resistance provided by *Mi-1* gene in tomato breaks down at soil temperatures above 28ºC in tomato-growing areas in many parts of the world. Therefore, planting time is important for tomato plants carrying *Mi-1* gene. These results showed that resistance did not break down in plants exposed to high soil temperatures before nematode inoculation. Therefore, the *Mi-1* gene-mediated resistance in tomato does not break down in seedling facilities, which are not usually infested with RKN, if the soil temperature in nursery is above 28ºC. In addition, results showed resistance break down in nematode-inoculated plants held at 32ºC for ≥48 h. The findings could help in the effective use of *Mi-1* gene and indicate the appropriate planting times of tomatoes bearing *Mi-1* gene for cultivation in fields prone to high soil temperatures (≥32ºC).

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