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Original article (Orijinal araştırma)

Reproduction of root-knot nematode isolates from the middle Black Sea Region of Turkey on tomato with *Mi-1.2* resistance gene[1]

Türkiye'nin Orta Karadeniz Bölgesi'nden elde edilen kök-ur nematodu izolatlarının *Mi-1.2* dayanıklılık geni taşıyan domateste üremesi

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Abstract

Research was conducted to evaluate the reproduction of 90 *Meloidogyne* isolates including *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (38 isolates), *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (4 isolates), *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (11 isolates) and *Meloidogyne luci* Carneiro et al., 2014 (37 isolates) (Tylenchida: Meloidogynidae) from the middle Black Sea Region of Turkey on susceptible and resistant tomato cultivars in a greenhouse with temperature controlled between 2013 and 2014. Galling index and reproduction factor of nematode isolates were assessed in a pot assay and resistance-breaking isolates were determined according to their reproduction index. Among the isolates, only two isolates of *M. luci* (Or-2 and Pr-1) had a similar galling index on susceptible and resistant tomato ($P \leq 0.05$). Four isolates of *M. luci* (Çr-19, Or-2, Pr-1 and Pr-2), two isolates of *M. arenaria* (A-7 and Sn-11) and an isolate of *M. incognita* (A-11) produced more eggs than the initial inoculum on resistant tomato. These root-knot nematode isolates, except two isolates of *M. arenaria*, were classified as resistance-breaking isolates having a reproduction index higher than 10%. These findings showed that the *Mi-1.2* gene confers resistance to *M. luci* but four isolates of *M. luci* could overcome this resistance. This is the first report for resistance-breaking isolates of *M. luci* in tomato.

Keywords: *Meloidogyne, Mi-1.2* gene, resistance, tomato, virulent

Öz

Araştırma, Türkiye'nin Orta Karadeniz Bölgesi'nden elde edilen *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (38 izolat), *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (4 izolat), *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (11 izolat) ve *Meloidogyne luci* Carneiro et al., 2014 (37 izolat) (Tylenchida: Meloidogynidae)'den oluşan 90 *Meloidogyne* izolatının hassas ve dayanıklı domates çeşitlerindeki üremesini değerlendirmek için 2013-2014 yılları arasında sıcaklık kontrollü bir serada yürütülmüştür. Nematod izolatlarının üreme faktörü ve urlanma indeksi saksı denemesi ile değerlendirilmiş ve üreme indeksi değerlerine göre dayanıklılığı kıran izolatlar belirlenmiştir. İzolatlar arasından sadece iki *M. luci* izolatı (Or-2 ve Pr-1) hassas ve dayanıklı domateste benzer bir urlanma indeksine sahiptir ($P \leq 0.05$). *M. luci*'nin dört izolatı (Çr-19, Or-2, Pr-1 ve Pr-2), *M. arenaria*'nın iki izolatı (A-7 ve Sn-11) ve *M. incognita*'nın bir izolatı (A-11), dayanıklı domateste başlangıç inokulumundan daha fazla yumurta meydana getirmiştir. Bu kök-ur nematodu izolatları, *M. arenaria*'nın iki izolatı hariç, %10'dan fazla üreme indeksine sahip olarak dayanıklılığı kıran izolatlar olarak sınıflandırıldı. Bu bulgular, *Mi-1.2* geninin *M. luci*'ye dayanıklılık sağladığını ancak dört izolatın bu dayanıklılığı kırabileceğini göstermiştir. İlk defa, domateste *M. luci'*nin dayanıklılığı kıran izolatları kayıt edilmiştir.

Anahtar sözcükler: *Meloidogyne*, *Mi-1.2* geni, dayanıklılık, domates, virülent

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Introduction

Root-knot nematodes (RKN), *Meloidogyne* spp., are polyphagous plant parasites and they are among the most destructive pests in protected vegetable cropping areas in different geographic regions of Turkey (Söğüt & Elekçioğlu, 2000; Devran & Söğüt, 2009; Akyazı & Ecevit, 2011; Aydınlı & Mennan, 2016; Uysal et al., 2017). While *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 and *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Meloidogynidae) are widely distributed throughout the country, *Meloidogyne ethiopica* Whitehead, 1968 was encountered only in the middle Black Sea coastal areas of Turkey (Aydınlı & Mennan, 2016). *Meloidogyne ethiopica* was detected in Turkey for the first time in tomato (*Solanum lycopersicum* L.) and cucumber (*Cucumis sativus* L.) greenhouses located in Samsun Province in 2009 (Aydınlı et al., 2013). In 2014, *Meloidogyne luci* Carneiro et al., 2014 was described as a new RKN species detected in vegetables, flowers and fruits in Brazil, Iran and Chile (Carneiro et al., 2014). This new species, very close to *M. ethiopica*, raised doubts about the accuracy of the earlier reported *M. ethiopica* isolates from Europe (Carneiro et al., 2014; Gerič Stare et al., 2017). Soon after, the Slovenian isolate known as the first record of *M. ethiopica* in Europe was identified as *M. luci* (Janssen et al., 2016). Recently, Gerič Stare et al. (2017) re-examined several isolates previously identified as *M. ethiopica* from different countries in Europe, including Turkey, and re-identified them as *M. luci*. Therefore, all the RKN isolates previously recorded as *M. ethiopica* from Turkey are now considered to be *M. luci*. To date, this nematode has been identified from Europe (Slovenia, Italy, Greece, Turkey and Portugal), America (Brazil, Chile and Guatemala) and Asia (Iran) (Conceição et al., 2012; Carneiro et al., 2014; Janssen et al., 2016; Gerič Stare et al., 2017; Maleita et al., 2018).

The management of RKN is generally practiced through four main methods, chemical treatment, cultural practices, biological control and host plant resistance (Nyczepir & Thomas, 2009). Among these methods, the use of resistance is the most convenient since it does not require special equipment and does not have any adverse effects on human health and the environment (Boerma & Hussey, 1992; Jacquet et al., 2005; Sorribas et al., 2005; Cortada et al., 2008; Verdejo-Lucas et al., 2009). Additionally, resistant plants can also be easily integrated with other control methods (Roberts, 1982). Resistant cultivars can efficiently suppress nematode reproduction; thus, they should be included in crop rotations to reduce nematode density in soils and to protect subsequent susceptible cultivars without extra cost (Mukhtar et al., 2013).

The *Mi-1.2* gene in tomato is one of the well-known resistance genes effective against three major RKN species (*M. arenaria, M. incognita* and *M. javanica*) (Verdejo-Lucas et al., 2009). Therefore, commercial tomato cultivars and rootstock cultivars with the *Mi-1.2* gene have been widely grown in protected vegetable production areas (Cortada et al., 2009). However, resistance-breaking or virulent populations can reproduce on resistant *Mi-1.2* plants and compromise the durability of the resistance against these nematodes (Verdejo-Lucas et al., 2009; Devran & Söğüt, 2010). Virulent nematodes can exist naturally in the field without previous exposure to resistant cultivars or through repeated culture of resistant tomatoes with the *Mi-1.2* gene (Roberts, 1995; Tzortzakakis & Gowen, 1996; Eddaoudi et al., 1997; Castagnone-Sereno, 2002). In many countries, wide geographical distribution of *Mi-1.2* virulent populations of *M. arenaria*, *M. incognita* and *M. javanica* have been reported (Riggs & Winstead, 1959; Prot, 1984; Kaloshian et al., 1996; Eddaoudi et al., 1997; Ornat et al., 2001; Tzortzakakis et al., 2005; Devran & Söğüt, 2010).

In a recent extensive survey, Aydınlı & Mennan (2016) observed RKN on tomato rootstocks in protected vegetable areas in the middle Black Sea Region of Turkey, where tomato is grown as a monoculture. Furthermore, *M. luci* (formerly known as *M. ethiopica*) was detected only on the weeds in greenhouses where resistant rootstocks or resistant tomato cultivars were grown. Based on these findings, researchers hypothesized that (i) nematode populations detected on rootstocks in tomato monocultures are *Mi-1.2* virulent and (ii) the *Mi-1.2* gene confers resistance to *M. luci*. Therefore, this study was conducted to assess the reproduction of 90 RKN isolates, obtained from the same region in Turkey, on resistant and susceptible tomato cultivars.

Materials and Methods

Nematode isolates

Ninety RKN isolates including *M. arenaria* (38), *M. incognita* (4), *M. javanica* (11) and *M. luci* (formerly reported as *M. ethiopica*, 37) from greenhouses located in the middle Black Sea Region of Turkey were collected and identified in a previous study (Aydınlı & Mennan, 2016) (Table 1). After reclassification of all *M. ethiopica* isolates reported from Europe (Gerič Stare et al., 2017), the identification of *M. ethiopica* isolates, reported in a previous study (Aydınlı & Mennan, 2016), was re-examined for esterase isozyme marker, the most distinguishing character between *M. ethiopica* and *M. luci*, and all the isolates were identified as *M. luci*. Additionally, the identification of several *M. luci* isolates, included in this study, was also confirmed by molecular analysis (Gerič Stare et al., 2017; Maleita et al., 2018).

Pure RKN isolates were obtained from single egg masses and maintained on susceptible tomato cv. Falcon plants (May Seed, Turkey) in pots in greenhouses with controlled temperature (24±2°C). These pot cultures were used to obtain nematode inoculum.

Pot assays

The study was conducted between 2013 and 2014 at University of Ondokuz Mayıs, Turkey. Reproduction of *Meloidogyne* isolates on a resistant tomato cv. Alsancak RN (Yuksel Seeds, Turkey) bearing the *Mi-1.2* gene was compared with a susceptible tomato cv. Beril (Rito Seeds, Turkey). Whether tomato cultivars carried the *Mi-1.2* resistance gene was assess using the molecular marker Mi23 and the presence of this gene in the resistant tomato cultivar was confirmed (Seah et al., 2007).

Tomato seedlings with 3-4 true leaves were transplanted singly into plastic pots containing 500 g sterilized sandy-loam soil and sand (2:1). Nematode eggs were obtained by rinsing the infected tomato roots from the pure nematode cultures using 10% commercial bleach solution (0.52% sodium hypochlorite) (Hussey & Barker, 1973). Five or 7 d after transplanting, for each of the 90 RKN isolates, five pots of each of the resistant and susceptible tomato cultivars were inoculated with 1000 RKN eggs (initial population density, Pi). Pots were arranged in a randomized block design and were grown in greenhouses at 24±2°C. Eight weeks after the inoculation, root systems were removed from the pots, washed with tap water, and rated for gall index (GI) using a 0-10 scale (Bridge & Page, 1980). Then, the roots were macerated in a blender with 20% commercial bleach solution for 30-40 s, the suspension poured onto 200 and 500 mesh sieves, and eggs were collected from the 500 mesh sieve for counting. Final population densities (Pf) of each nematode isolate on resistant and susceptible tomato cultivars were determined. The reproduction factor (Rf = Pf/Pi) and reproduction index (RI = Pf on the resistant cultivar/Pf on susceptible cultivar X 100) were calculated (Ornat et al., 2001; Cortada et al., 2008).

The resistance level of tomato cultivar containing *Mi-1.2* gene to each RKN isolate was categorized according to the RI as highly resistant (RI < 10%), moderately resistant (10% ≤ RI < 50%) or susceptible (RI ≥ 50%) (Cortada et al., 2009). Isolates having a RI value higher than 10% were considered as resistance-breaking and those isolates that caused moderately resistant or susceptible reactions on this cultivar were classified as partially virulent or virulent, respectively.

Data analysis

All data were analyzed with SAS statistical software. Each *Meloidogyne* species was evaluated separately. GI and Rf on resistant plants were log-transformed [log10(x+1)] before analysis. Data from both resistant and susceptible plants were subjected to analysis of variance. Differences between isolates from the same nematode species were evaluated using the Duncan's multiple range test at P ≤ 0.05 significance level. GI and Rf of each RKN isolate on susceptible and resistant cultivars were compared with t-test at P ≤ 0.05 significance level (Devran & Söğüt, 2010).

Results

Molecular marker Mi23 indicated that cv. Alsancak RN is a hybrid and heterozygous for the *Mi* locus (*Mi/mi*) with 380 and 430 bp DNA fragments, whereas susceptible tomato cultivar displayed a single DNA fragment with 430 bp, confirming the absence of the *Mi-1.2* gene (*mi/mi*).

The reproduction of the RKN isolates on the susceptible and resistant cultivars was evaluated within each nematode species (Table 1). As expected, all isolates reproduced on the susceptible tomato cultivar (Pf of 6 to 47.8 times greater than Pi) and had greater Rf values on susceptible than on the resistant tomato cultivar ($P < 0.05$).

Meloidogyne arenaria isolates had lower GI on the resistant than on the susceptible tomato cultivar ($P < 0.05$). However, *M. arenaria* A-7 and Sn-11 had a Rf higher than 1.0 on resistant tomato, but the RI were 4.77 and 4.13%, respectively. Although the highest RI of *M. arenaria* was 5.67% for A-12, this isolate on the resistant cultivar produced lower number of eggs than the Pi. Overall, the tomato cultivar with *Mi-1.2* gene displayed high level of resistance (RI < 10%) against all *M. arenaria* isolates.

On the susceptible cultivar, of the four *M. incognita* isolates A-11 had significantly lower Rf than the other isolates ($P < 0.05$). However, this isolate showed the highest GI (4.8) and Rf (4.8) on the resistant tomato cultivar in contrast to the other *M. incognita* isolates that were unable to reproduce on the resistant tomato. Similarly, the resistant tomato cultivar was highly resistant (RI < 10%) to all *M. incognita*, except for A-11 (RI = 15.1%) that caused a moderately resistant reaction on this cultivar.

GI values of the *M. javanica* isolates on the resistant cultivar ranged from 0 to 2.8 and the nematodes did not reproduce (Rf < 1) on this cultivar indicating high level of resistance (RI < 10%).

For four isolates of *M. luci* (Çr-19, Or-2, Pr-1 and Pr-2) on the resistant cultivar, the Rf values were >1, ranging from 3.6 to 12.6 times greater than the Pi. Similarly, on the resistant tomato, the GI values of these four *M. luci* isolates were the highest ranging from 5.6 to 7.0 which did not significantly differ ($P < 0.05$). Moreover, the GI values of two isolates (Or-2 and Pr-1) were not significantly different on the resistant and susceptible tomato cultivars. Remarkable differences were detected for the RI values for *M. luci*, ranging from 0 to 51.2%. Isolate Or-2 had the highest RI (51.2%) on the cv. Alsancak, with *Mi-1.2* gene, responding as susceptible. However, Alsancak was moderately resistant (10% ≤ RI < 50%) to three isolates (RI: Pr-2 = 46.4%, Pr-1 = 21.1% and Çr-19 = 16.0%) and highly resistant (RI < 10%) to the remaining (33) *M. luci* isolates.

Table 1. Gall index (GI), reproduction factor (Rf) and reproduction index (RI) of *Meloidogyne* spp. isolates on susceptible (S) and resistant (R) tomato cultivars, 8 weeks after inoculation of 1000 eggs/plant in a pot assay conducted in a temperature-controlled greenhouse (24±2°C)*

Code[a]	*Meloidogyne* species	Host Plant	GI (0-10)[b]		Rf[c]		RI%[d]
			S	**R**	**S**	**R**	
Er-1	*M. arenaria*	Cucumber	7.2±0.20 d-f	2.2±0.20 b-f	18.8±1.91 m-o	0.31±0.12 de	1.65
Er-2			7.0±0.00 e-g	2.4±0.24 b-f	15.8±1.24 o-q	0.22±0.08 d-f	1.39
Er-4			7.6±0.24 b-e	2.4±0.24 b-f	33.4±1.54 e-g	0.21±0.07 d-f	0.63
Er-5			7.6±0.24 b-e	1.6±0.24 e-h	24.0±1.73 j-l	0.12±0.04 e-k	0.50
Er-6			7.8±0.20 a-d	0.6±0.24 i-k	16.8±1.36 o-q	0.03±0.02 h-k	0.18
A-5			8.0±0.00 a-c	1.8±0.20 d-g	25.6±2.20 i-k	0.11±0.03 f-k	0.43
A-7			8.2±0.20 ab	4.0±0.00 a	30.8±2.22 f-h	1.47±0.11 a	4.77

Table 1. Continued

Code[a]	*Meloidogyne* species	Host Plant	GI (0-10)[b]		Rf[c]		RI%[d]
			S	**R**	**S**	**R**	
A-8			7.8±0.20 a-d	3.0±0.00 a-d	28.2±1.66 h-j	0.64±0.07 c	2.27
A-12			7.8±0.20 a-d	3.2±0.20 a-c	16.4±1.03 o-q	0.93±0.12 b	5.67
A-13			6.0±0.32 i	0.6±0.24 i-k	12.8±0.97 p-s	0.08±0.03 f-k	0.63
A-17			8.2±0.20 ab	3.6±0.24 ab	37.4±1.29 de	0.68±0.13 c	1.82
A-18			6.4±0.24 g-i	0.8±0.20 h-j	9.6±0.75 rs	0.06±0.03 f-k	0.63
Sn-7			8.4±0.24 a	2.2±0.20 b-f	35.2±1.59 d-f	0.16±0.02 d-j	0.45
Sn-9			8.4±0.24 a	2.0±0.00 c-f	47.6±1.03 a	0.12±0.01 e-k	0.25
Sn-11			8.2±0.20 ab	3.2±0.20 a-c	29.8±1.53 g-i	1.23±0.05 a	4.13
Om-2			7.2±0.20 d-f	0.2±0.20 k	18.8±1.43 m-o	0.02±0.02 i-k	0.11
B-3			7.8±0.20 a-d	1.4±0.24 f-h	12.4±1.03 q-s	0.20±0.07 d-g	1.61
B-17			7.4±0.24 c-f	0.2±0.20 k	9.8±0.66 rs	0.00±0.00 k	0.00
B-19			7.6±0.24 b-e	0.6±0.24 i-k	21.8±2.44 k-n	0.11±0.05 f-k	0.50
B-20			8.2±0.20 ab	0.6±0.24 i-k	30.6±1.44 f-h	0.11±0.04 f-k	0.36
B-27			7.0±0.00 e-g	0.4±0.24 jk	8.2±0.58 s	0.01±0.01 jk	0.12
B-30			6.8±0.20 f-h	0.4±0.24 jk	17.3±0.97 n-p	0.03±0.02 h-k	0.17
Çr-1			8.0±0.00 a-c	2.2±0.20 b-f	10.6±1.08 rs	0.21±0.07 d-f	1.98
Çr-23			7.0±0.00 e-g	1.6±0.24 e-h	8.6±0.68 rs	0.10±0.03 f-k	1.16
Çr-30			7.8±0.20 a-d	0.2±0.20 k	42.0±1.45 bc	0.02±0.02 i-k	0.05
Çr-33			7.8±0.20 a-d	0.4±0.24 jk	17.4±1.57 n-p	0.05±0.03 f-k	0.29
Çr-35			7.6±0.24 b-e	0.4±0.24 jk	26.8±1.36 h-j	0.00±0.00 k	0.00
Ço-2			8.2±0.20 ab	1.0±0.00 g-i	38.8±1.59 cd	0.33±0.05 d	0.85
Ço-3			8.0±0.00 a-c	0.8±0.20 h-j	23.4±1.86 j-m	0.18±0.05 d-i	0.77
Ço-6			7.6±0.24 b-e	1.0±0.00 g-i	43.8±1.46 ab	0.17±0.08 d-j	0.39
Çr-26		Melon	7.8±0.20 a-d	0.6±0.24 i-k	25.2±1.69 i-k	0.06±0.02 f-k	0.24
Er-8		Nightshade	7.6±0.24 b-e	2.6±0.24 a-e	17.2±2.06 n-q	0.19±0.05 d-h	1.10
A-4			7.8±0.20 a-d	1.6±0.24 e-h	23.4±1.94 j-m	0.15±0.05 e-k	0.64
Tk-1			6.8±0.20 f-h	0.8±0.20 h-j	36.3±1.46 de	0.08±0.02 f-k	0.22
Çr-28			8.0±0.00 a-c	1.6±0.24 e-h	44.2±1.43 ab	0.15±0.05 e-k	0.34
A-9		Tomato[Rt]	7.4±0.24 c-f	2.8±0.20 a-e	19.4±1.50 l-o	0.56±0.08 c	2.89
A-15		Tomato	6.2±0.20 hi	0.6±0.24 i-k	13.2±1.32 p-r	0.07±0.03 f-k	0.53
Pr-7			6.4±0.24 g-i	0.4±0.24 jk	33.7±1.50 e-g	0.04±0.03 g-k	0.12

Table 1. Continued

Code[a]	*Meloidogyne* species	Host Plant	GI (0-10)[b]		Rf[c]		RI%[d]
			S	**R**	**S**	**R**	
Çr-20	*M. incognita*	Cucumber	8.2±0.20 a	1.2±0.20 b	38.2±0.86 a	0.00±0.00 b	0.00
Pr-4			6.8±0.20 b	0.6±0.24 c	35.9±1.03 a	0.06±0.03 b	0.18
Tr-14		Pepper	7.6±0.24 a	0.0±0.00 d	36.0±1.14 a	0.00±0.00 b	0.00
A-11		Tomato[Rt]	8.2±0.20 a	4.8±0.20 a	31.8±0.97 b	4.80±0.42 a	15.09
Sn-4	*M. javanica*	Cucumber	8.0±0.00 ab	2.8±0.20 a	44.8±0.86 ab	0.48±0.05 b	1.07
Sn-5			7.0±0.00 e	2.2±0.20 ab	35.4±1.21 d	0.21±0.03 cd	0.60
Tr-8			7.4±0.24 c-e	0.8±0.20 cd	42.3±0.90 b	0.09±0.02 d-f	0.20
B-1			7.2±0.20 de	1.4±0.24 bc	24.2±1.16 f	0.24±0.05 cd	0.98
B-7			8.2±0.20 a	2.6±0.24 a	45.8±1.39 a	0.81±0.14 a	1.76
B-18			7.6±0.24 b-d	0.4±0.24 de	38.6±1.03 c	0.16±0.10 c-e	0.41
Çr-41			7.6±0.24 b-d	0.2±0.20 e	31.5±0.94 e	0.02±0.02 e-f	0.07
Ço-1			8.0±0.00 ab	0.0±0.00 e	47.8±1.02 a	0.00±0.00 f	0.00
B-6		Eggplant	7.8±0.20 a-c	0.0±0.00 e	10.2±0.86 g	0.00±0.00 f	0.00
B-26		Tomato	8.0±0.00 ab	1.8±0.20 ab	47.6±1.21 a	0.25±0.05 c	0.52
Çr-27			8.2±0.20 a	0.8±0.20 cd	38.4±0.93 cd	0.10±0.03 c-f	0.26
Sn-3	*M. luci*	Cucumber	7.6±0.24 a-d	1.2±0.20 c-e	21.4±1.50 f-i	0.07±0.03 g-i	0.32
Sn-12			7.8±0.20 a-c	0.4±0.24 fg	21.4±1.36 f-i	0.03±0.02 hi	0.15
Tk-2			7.4±0.24 b-e	0.2±0.20 g	7.4±0.53 rs	0.03±0.03 hi	0.43
Al-2			7.6±0.24 a-d	0.4±0.24 fg	14.6±1.21 k-o	0.05±0.03 g-i	0.36
Al-3			8.0±0.00 ab	0.2±0.20 g	23.4±1.63 e-g	0.02±0.02 hi	0.08
Al-4			7.4±0.24 b-e	0.4±0.24 fg	9.8±1.07 p-s	0.04±0.03 hi	0.43
Om-1			7.4±0.24 b-e	0.0±0.00 g	25.6±1.50 de	0.00±0.00 i	0.00
Tr-1			8.0±0.00 ab	0.4±0.24 fg	29.8±1.36 c	0.03±0.02 hi	0.11
Tr-2			7.6±0.24 a-d	0.4±0.24 fg	29.8±1.69 c	0.08±0.05 g-i	0.27
Tr-3			6.6±0.24 fg	0.4±0.24 fg	9.2±0.86 q-s	0.00±0.00 i	0.00
Tr-5			8.0±0.00 ab	0.4±0.24 fg	23.4±0.81 e-g	0.06±0.04 g-i	0.26
Tr-13			8.0±0.00 ab	0.4±0.24 fg	36.2±1.20 b	0.14±0.09 g-i	0.39
Çr-3			7.6±0.24 a-d	1.2±0.20 c-e	6.9±0.48 s	0.05±0.03 g-i	0.67
Çr-5			7.8±0.20 a-c	0.2±0.20 g	27.4±1.89 cd	0.02±0.02 hi	0.08
Çr-7			7.6±0.24 a-d	2.2±0.20 bc	20.2±1.77 g-j	0.37±0.06 ef	1.81
Çr-9			8.0±0.00 ab	2.4±0.24 b	30.0±1.22 c	0.48±0.14 e	1.59

Table 1. Continued

Code[a]	*Meloidogyne* species	Host Plant	GI (0-10)[b]		Rf[c]		RI%[d]
			S	**R**	**S**	**R**	
Çr-10			7.8±0.20 a-c	0.6±0.24 e-g	9.8±0.72 p-s	0.11±0.05 g-i	1.12
Çr-11			8.0±0.00 ab	0.6±0.24 e-g	21.8±1.07 e-h	0.07±0.03 g-i	0.32
Çr-19			7.4±0.24 b-e	5.6±0.24 a	42.5±0.91 a	6.8±0.83 c	16.00
Çr-25			8.00±0.00 ab	0.2±0.20 g	15.2±0.86 k-n	0.03±0.03 hi	0.17
Çr-34			7.6±0.24 a-d	1.8±0.20 b-d	6.0±0.35 s	0.19±0.04 f-h	3.23
Çr-36			7.0±0.00 d-f	0.2±0.20 g	12.0±1.22 n-q	0.01±0.01 i	0.08
Çr-39			7.0±0.00 d-f	1.0±0.00 d-f	13.4±1.21 m-p	0.00±0.00 i	0.00
Çr-40			8.2±0.20 a	0.6±0.24 e-g	17.8±1.85 i-l	0.07±0.04 g-i	0.42
Pr-1[e]			6.4±0.24 gh	5.8±0.20 a	17.1±1.04 j-m	3.60±0.58 d	21.05
Pr-2			7.2±0.20 c-e	5.6±0.24 a	22.0±1.21 e-h	10.20±0.75 b	46.36
Om-3		Eggplant	7.8±0.20 a-c	0.2±0.20 g	11.2±0.86 o-r	0.00±0.00 i	0.00
Tk-4		Nightshade	6.0±0.00 h	1.4±0.24 b-d	9.7±0.98 p-s	0.15±0.05 g-i	1.53
Tr-19			8.0±0.00 ab	0.4±0.24 fg	14.6±1.44 k-o	0.03±0.03 hi	0.19
Çr-2			6.8±0.20 e-g	0.0±0.00 g	19.4±1.21 h-j	0.00±0.00 i	0.00
Çr-12			7.6±0.24 a-d	1.4±0.24 b-d	13.0±1.00 n-q	0.09±0.04 g-i	0.68
Çr-15			8.0±0.00 ab	0.6±0.24 e-g	22.2±1.16 e-h	0.09±0.04 g-i	0.42
Or-2[e]			7.4±0.24 b-e	7.0±0.32 a	24.6±0.53 d-f	12.60±1.02 a	51.22
Tr-18		Tomato	7.8±0.20 a-c	0.4±0.24 fg	18.4±0.93 h-k	0.07±0.04 g-i	0.38
B-32			7.8±0.20 a-c	1.8±0.20 b-d	14.8±1.55 k-o	0.22±0.03 fg	1.50
Çr-24			7.8±0.20 a-c	0.4±0.24 fg	14.8±1.36 k-o	0.04±0.02 hi	0.26
Or-1			6.8±0.20 e-g	0.2±0.20 g	13.9±0.43 l-o	0.02±0.02 hi	0.13

* Data are means of five replicates±standard errors. Statistical analyses of the data were based on log10(x+1) transformed data. Root-knot nematode species were analyzed separately and grouped to form clusters within each species. For each root-knot nematode species, values in the same column followed by the same letter are not significantly different according to Duncan's multiple range test at $P < 0.05$;

S: Beril F1 (Rito Seeds, Turkey), R: Alsancak RN F1 (Yuksel Seeds, Turkey);

[a] Letters in the isolate codes indicate the location of the isolates collected from Black Sea Region of Turkey. A: Amasya, Al: Alaçam, B: Bafra, Ço: Çorum, Çr: Çarşamba, Er: Erbaa, Om: Ondokuzmayıs, Or: Ordu, Pr: Perşembe, Sn: Sinop, Tk: Tekkeköy, and Tr: Terme

[b] Based on a scale from 0 (none) to 10 (dead plants);

[c] final population density (Pf) / initial population density (Pi);

[d] (Pf on resistant cultivar / Pf on susceptible cultivar) x 100;

[e] GI of nematode isolate on susceptible and resistant cultivars are not significantly different according to t-test;

[Rt] Rootstock.

Discussion

The potential use of a resistant cultivar for nematode control depends on nematode virulence. This study reports the response of the resistant tomato cv. Alsancak, heterozygous for the *Mi* locus (*Mi/mi*), against 90 RKN isolates compared to that on the susceptible tomato cv. Beril. The resistant tomato cultivar exhibited wide variations in its response to RKN isolates, which was highly resistant to 85 isolates but moderately resistant to four isolates and susceptible to one isolate.

One *M. incognita* (A-11) and four *M. luci* (Çr-19, Or-2, Pr-1 and Pr-2) with RI > 10% were considered as resistance-breaking isolates and characterized as partially virulent or virulent according to host reaction of resistant cv. Alsancak based on RI. Among these isolates, only *M. luci* Or-2 isolate showed RI higher than 50% and was classified as virulent, whereas other isolates were partially virulent because of the moderately resistant response of the cv. Alsancak to these isolates (10% ≤ RI < 50%). However, all isolates of the *M. arenaria* and *M. javanica* were considered as avirulent, despite *M. arenaria* A-7 and Sn-11 isolates on resistant cultivar reproduced without substantial increases (Rf close to 1.0). *Meloidogyne arenaria* A-9 and *M. incognita* A-11 were expected to be resistance-breaking isolates as they were isolated from tomato rootstocks cv. King Kong RZ F1 (Rijk Zwaan) and cv. Kemerit RZ F1 (Rijk Zwaan), respectively, both presumed to have the *Mi-1.2* gene. Nevertheless, in the pot assays, the *M. incognita* A-11 reproduced in the resistant cultivar, but the *M. arenaria* A-9 did not (Rf < 1). Similarly, Sorribas & Verdejo-Lucas (1994) reported that three isolates of *Meloidogyne* species reproduced in resistant tomato in the field, and were considered as resistance-breaking isolates, but two of these isolates did not reproduce in resistant tomato in greenhouse assays. Eddaoudi et al. (1997) also reported that an isolate obtained from resistant tomato plant in the field did not reproduce in resistant cultivars in pot assays. Probably, there is more than one reason why an isolate that reproduced in resistant tomato in the field or greenhouse did not reproduce on resistant tomato under temperature-controlled conditions (<28°C). The *Mi-1.2* gene efficiency is lost at temperatures above 28°C (Dropkin, 1969; Ammati et al., 1986). Recently, Özalp & Devran (2018) reported that the resistance controlled by *Mi-1.2* gene in tomato plants held at 32ºC soil temperature for ≥48 h lost its effect. Therefore, nematodes will be to reproduce on resistant tomato plants in a greenhouse during the summer period when the temperature in the pots exceeds 28°C. An isolate of *M. javanica* did not reproduce in resistant cultivars in a controlled environment (soil temperature 22-24°C) and registered as avirulent but it reproduced under greenhouse conditions with soil temperatures ranging between 22°C to 42°C (Tzortzakakis & Gowen, 1996). Genetic background of tomato cultivars and rootstocks bearing the *Mi-1.2* resistance gene may cause differences on the virulence level of a nematode population (Roberts & Thomason, 1986; Jacquet et al., 2005; Cortada et al., 2009). Another reason for the difference in virulence level of a nematode isolate in a greenhouse compared to controlled conditions might be related to the nematode inoculum density. Within an avirulent population, low numbers of virulent nematodes can be present and consequently at a high density of nematodes the number of virulent individuals will increase resulting in high levels of infection on resistant roots (Tzortzakakis & Gowen, 1996; Castagnone-Sereno et al., 2007; Verdejo-Lucas et al., 2013). Moreover, Tzortzakakis et al. (2008) noted that the assessment of the virulence of RKN isolates for resistant cultivars in pot assays may not always be reliable because limited quantity of inoculum used in these assays may reduce the possibility to detect the virulent individuals with low densities within a population. It is therefore important to emphasize that repeated culture of resistant tomato cultivars in the same field may increase the proportion of virulent individuals (Sorribas et al., 2005). Virulent populations may be selected from an initial avirulent population because of selection pressure (Jarquin-Barberena et al., 1991; Castagnone-Sereno et al., 2007). Rotation of susceptible and resistant tomato cultivars instead of continuously cultivation of resistant cultivars in the same field would delay the occurrence of virulent populations and provide stability of the plant resistance (Castagnone-Sereno et al., 2007; Djian-Caporalino et al., 2011).

A large number of *M. luci* isolates were tested against the resistant tomato cv. Alsancak and the results showed that *Mi-1.2* gene confers resistance to *M. luci*. Previously, Strajnar & Širca (2011) reported that tomato cv. Venezia with *Mi-1.2* resistance gene was resistant to a Slovene *M. luci* isolate. Similarly, attempts to establish two Greek *M. luci* isolates on resistant tomato cv. Silvana by Conceição et al. (2012) failed. However, in this study, we identified a *M. luci* isolate (Or-2) that reproduces (51.2% RI) in the resistant cv. Alsancak. Or-2 was obtained from the roots of a weed (*Solanum nigrum* L.) growing in a greenhouse with grafted tomato. Notably, the roots of the grafted tomato plants near this weed had low numbers of galls. Although this isolate had an avirulent reaction in tomato rootstocks grown in a greenhouse, this isolate was classified as virulent based on the pot assay described in this study. Though no information exists on the tomato rootstock used in the greenhouse, it is likely that the genetic background of the rootstock was different from that of the resistant cultivar used in our pot assay. The other resistance-breaking isolates of *M. luci* (Çr-19, Pr-1 and Pr-2) were collected from cucumbers planted during two summer cropping cycles in greenhouses and the cropping history before those two years is unknown. Therefore, it is likely that these three *M. luci* isolates are naturally virulent to the *Mi-1.2* gene.

Mi-1.2 virulent isolates of *M. incognita* and *M. javanica* were reported for the first time in the Mediterranean Region of Turkey by Devran & Söğüt (2010). However, resistance-breaking isolates of *M. arenaria* were not recorded in Turkey (Devran & Söğüt, 2010; Özarslandan & Elekçioğlu, 2010). This study presents the first report of *Mi-1.2* resistance-breaking isolates of *M. luci*. Together with previous findings, it is concluded that *M. luci* is a potential threat for economically important crops grown in vegetable cropping areas (Aydınlı et al., 2013; Aydınlı & Mennan, 2016; Aydınlı, 2018). Thus, further research should be conducted to elucidate the mechanisms related to virulence of this nematode species in resistant cultivars.

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