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Resistance Response of Tomato Cultivars and Rootstocks Carrying the *Mi-1.2* Gene to Isolates of *Meloidogyne arenaria*, *M. incognita*, and *M. javanica* at Two Different Growing Periods

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Abstract

The reaction of four tomato cultivars and four rootstocks, carrying the *Mi-1.2* gene, to different isolates of *Meloidogyne arenaria* (4 isolates), *M. incognita* (2 isolates), and *M. javanica* (2 isolates) were determined in pot experiments conducted in a non-temperature controlled greenhouse. Tomato cultivars and rootstocks were screened by PCR-based co-dominant SCAR marker Mi23 for the zygosity states of the *Mi-1* locus. All resistant tomato cultivars and the rootstock 'Comfort' were classified as heterozygous resistant (*Mimi*), whereas the rootstocks 'Arazi', 'Beaufort', and 'Kingkong' were homozygous resistant (*MiMi*) at the *Mi-1* locus. The response of resistant genotypes to isolates of *Meloidogyne* was assessed 8 and 16 weeks after inoculation with 3,000 nematode eggs for short and long growing periods, respectively. Nematode reproduction on resistant cultivars was similar or higher than on resistant rootstocks for all tested *Meloidogyne* isolates in the short period experiment ($p < 0.05$), but not in the long period experiment. In both experiments, all tomato cultivars and rootstocks responded as highly resistant (reproduction index < 10%) to all tested *Meloidogyne* isolates except for *M. incognita* A-11. Of the resistant genotypes inoculated with *M. incognita* A-11, in both experiments, the cultivars 'Crisol' and 'Adamset' were moderately resistant, whereas the rootstocks 'Beaufort' and 'Comfort' were highly resistant. 'Alsancak', 'Esin', 'Arazi', and 'Kingkong' showed reduced resistance in the long growing period and responded as moderately resistant to *M. incognita* A-11. These findings showed that the resistance response of tomato cultivars and rootstocks to *Meloidogyne* isolates varies depending on the isolates of *Meloidogyne* or the length of the growing period.

Additional key words: management, PCR, reproduction, root-knot nematodes, *Solanum lycopersicum* L.

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Introduction

Tomatoes (*Solanum lycopersicum* L.), members of the Solanaceae family, are typical components of human nutrition and used extensively in diverse cuisines in the world with annual production of 177 million tons in 2016 (FAO, 2018). Turkey is the fourth largest tomato producer in the world, which accounts for 7.2% (12.7 million tons) of the total annual production in 2016 (FAO, 2018).

Root-knot nematodes (RKN), *Meloidogyne* spp., are a major threat for tomato production due to their short life cycles and high reproductive rates (Trudgill and Blok, 2001). They have high damage potential and economic impact on tomato production especially in protected cultivation because of intensive cropping with a short fallow period, as well as suitable soil conditions under these production systems that ensure a favorable environment for high nematode population growth on susceptible cultivars (Lopez-Gomez et al., 2015). Therefore, effective nematode control is required to improve crop productivity in these systems. Although nematicides have successfully controlled RKN populations, increasing human and environmental health concerns resulted in the restrictions on broad-spectrum nematicides. These limitations have contributed to an increased interest in the use of resistant tomato cultivars as an alternative control measure (Ornat et al., 2001; Devran et al., 2010). In addition, this increased interest in the use of resistance is because growing resistant plants/cultivars, instead of susceptible ones, does not require any additional farming practices (Lopez-Perez et al., 2006; Cortada et al., 2009; Devran et al., 2010).

RKN resistance in tomato cultivars and rootstocks is mediated by the single dominant *Mi-1.2* gene that was introgressed from a wild tomato species, *Solanum peruvianum* (Smith, 1944). Different molecular markers have been developed to detect the presence of this gene, which is located on the short arm of chromosome 6 (Williamson et al., 1994; El Mehrach et al., 2005; Seah et al., 2007). Furthermore, these markers indicate the Mi allelic conditions (*MiMi*, *Mimi*, and *mimi*) at the gene locus (Cortada et al., 2008; Devran et al., 2013). Plant genetic background has a major effect in the variability of the resistance response to nematodes (Jacquet et al., 2005; Lopez-Perez et al., 2006; Cortada et al., 2009; Verdejo-Lucas et al., 2009). Several authors suggested a possible dosage effect of the *Mi-1.2* gene that is more effective against the nematode in homozygous (*MiMi*) than heterozygous (*Mimi*) conditions (Tzortzakakis et al., 1998; Jacquet et al., 2005). Additionally, inter- and intra-specific genetic variability of *Meloidogyne* isolates contributes to the variation of the *Mi-1.2* response (Cortada et al., 2009). Therefore, *Mi-1.2*-mediated resistance is strongly influenced by the interaction between plant genotype and nematode isolate (Jacquet et al., 2005). Another major influence on the performance of the *Mi-1.2* gene is temperature. The efficacy of *Mi-1.2*-mediated resistance is reduced at soil temperatures above 28°C (Dropkin, 1969; Devran et al., 2010). However, daily fluctuations in soil temperatures with intermittent peaks above 28°C did not endanger the resistance (Araujo et al., 1982a; Verdejo-Lucas et al., 2013). The response of *Mi-1.2* gene exposed to high temperature was changed according to the length of the high temperature period, heat intensity, or their interaction (Verdejo-Lucas et al., 2013). Furthermore, resistance was affected by the timing of the high temperature period as it was lost when applied at the beginning of the experiment (young plants) shortly before or after nematode inoculation (Dropkin, 1969; Carvalho et al., 2015).

Meloidogyne-resistant tomato cultivars and rootstocks have been widely used in many greenhouses globally. Therefore, it is essential to explore the response of resistant tomato genotypes to RKN species in non-temperature controlled greenhouses for the development of effective control strategies. Although many studies on the response of tomato cultivars and rootstocks carrying the *Mi-1.2* gene against different isolates of *Meloidogyne* species have been reported (Ammati et al., 1986; Tzortzakakis et al., 1998; Ornat et al., 2001; Molinari and Caradonna, 2003; Lopez-Perez et al., 2006; Cortada et al., 2008, 2009; Devran et al., 2010; Verdejo-Lucas et al., 2013; Carvalho et al., 2015), little is known about the variation in the resistance response of tomato cultivars and rootstocks to *Meloidogyne* isolates at different growing periods (Cortada et al., 2008, 2009). The objectives of this study were to identify the *Mi-1.2* gene zygosity form in four tomato cultivars and four tomato rootstocks by PCR-based co-dominant SCAR marker Mi23, and to determine the reaction of these tomato

genotypes to eight different local isolates of *M. arenaria*, *M. incognita,* and *M. javanica* in pot experiments conducted in a non-temperature controlled greenhouse for short and long growing periods.

Materials and Methods

Nematode Isolates

Eight RKN isolates including four of *M. arenaria* (Er-4, A-7, Sn-11, and B-17), two of *M. incognita* (Pr-4 and A-11) and two of *M. javanica* (Ço-1 and Çr-27) were used in this study. The cultures of these isolates, collected in the commercial greenhouses in the Middle Black Sea Region of Turkey, were initiated from single egg masses and species were identified in a previous study (Aydınlı and Mennan, 2016). These isolates were maintained on nematode-susceptible tomato cv. Falcon in pots. The species identification of these isolates was confirmed using esterase phenotypes (Esbenshade and Triantaphyllou, 1985) before the pot experiments for bioassay were conducted.

Plant Materials

Tomato seedlings were purchased from a commercial supplier of vegetable seedlings (Olympus Fide, Antalya, Turkey). Five tomato cultivars and four rootstocks were used in the experiments (Table 1). Tomato 'Barbaros' was used as a nematode-susceptible control and also was grafted onto rootstocks.

Table 1. Origins and resistances of tomato cultivars and rootstocks used in this study

Tomato	Seed Company	Resistance[z]
Rootstocks		
Arazi	Sygenta Seeds	HR: ToMV 0-2/Ff A-E/Fol 1-2/For/Va IR: PI/ Ma/Mi/Mj
Beaufort	De Ruiter	HR: ToMV/Fol:0,1/For/PI/Va/Vd/Ma/Mi/Mj
Comfort	Tolya Seeds	HR: ToMV/Fol:0,1/Va/Vd/For/Pl/Ma/Mi/Mj
Kingkong	Rijk Zwaan	HR: ToMV:0-2/Fol:0,1/For/Pl/Va:0/Vd:0 IR: Ma/Mi/Mj
Cultivars		
Adamset	Sygenta Seeds	HR: TSWV/For/Va IR: Ma/Mi/Mj
Alsancak	Yüksel Seeds	HR: ToMV/Va/Vd/Fol: 0-1/Cf-5 IR: Ma/Mi/Mj
Barbaros	Seven Brothers Seeds	ToMV, Va, Fol: 0,1, TSWV
Crisol	Semillas Fitó	HR: ToMV/TSWV/Fol:0,1/Vd/Mi
Esin	Zeraim Gedera	HR: Vd/Fol/ToMV/TSWV IR: Ma/Mi/Mj

[z]Information was obtained from product catalogues or the website of the seed companies. HR: high resistance; IR: intermediate resistance; ToMV: *tomato mosaic virus*; TSWV: *tomato spotted wilt virus*; Va: *Verticillium albo-altum*; Vd: *V. dahliae*; Fol:0,1: *Fusarium oxysporum* race 0 and 1; For: *Fusarium oxysporum* f. sp. *radicis-lycopersici*; Cf: *Cladosporium fulvum*; Ff: *Fulvia fulva*; PI: *Pyrenochaeta lycopersici*; Ma: *Meloidogyne arenaria*; Mi: *M. incognita*; Mj: *M. javanica*.

Detection of *Mi* Gene

To assess whether test plants carried the *Mi-1.2* resistance gene and the homozygous or heterozygous condition for the *Mi* locus, a PCR study was performed using SCAR primers Mi23F (5'-TGG AAA AAT GTT GAA TTT CTT TTG-3') and Mi23R (5'- GCA TAC TAT ATG GCT TGT TTA CCC-3') specifically designed for this gene in tomato (Seah et al., 2007). DNA was extracted from young leaf tissue and root tissue of non-grafted and grafted seedlings, respectively, using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions.

PCR reactions were performed in a final volume of 25 μL containing 20 ng template DNA, 1X Taq buffer with KCl, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 μM of each primer, and 1.25 U of Taq DNA Polymerase (Thermo Scientific, Waltham, MA, USA). PCR was conducted with a T-100 Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA) and amplification conditions comprised an initial denaturation step at 94°C for 3 min, followed by 35 cycles of 30 s at 94°C, 30 s at 56°C, and 1 min at 72°C, with a final extension for 8 min at 7°C. PCR products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide and photographed by the gel documentation system, G:BOX F3 (Sygene, Cambridge, UK).

Bioassay

Experiments were conducted to evaluate the resistance response of tomato cultivars and rootstocks to different *Meloidogyne* species and isolates in short (8 weeks) and long (16 weeks) growing periods in a non-temperature controlled greenhouse (17-44°C). Seedlings were transplanted singly into plastic pots containing approximately 500 cm^3 and 1,500 cm^3 of sterilized sandy soil for short period and long period experiments, respectively, and allowed to grow for 1 week before nematode inoculation. Eggs of *Meloidogyne* isolates were obtained by shaking the infected tomato roots in a 0.5% NaOCl solution for 3 min (Hussey and Barker, 1973). The suspension containing the eggs and roots was poured onto 200 and 500 mesh sieves, and eggs were collected from the 500 mesh sieve. Egg suspensions were quantified and used as inocula. Plants were inoculated with 3,000 eggs (Pi) per plant by pipetting the egg suspension into three holes made in the soil around the plant (28 April). Each nematode isolate-plant genotype combination was replicated five times for both experiments. Pots were placed on greenhouse benches according to a randomized block design. The experiments were terminated 8 weeks (22 June) and 16 weeks (17 August) after nematode inoculation for the short and long growing periods, respectively. The root systems were removed from the pots, washed with tap water, and rated for gall index (GI) using a 0-10 scale (Bridge and Page, 1980). Then, the roots were macerated in a blender with a 1% NaOCl solution three times for 15 seconds with 30 seconds intervals, the suspension poured onto 200 and 500 mesh sieves, and eggs were collected from the 500 mesh sieve for determining the final population density (Pf) of each combination. The reproduction factor (Rf = Pf/Pi) and reproduction index (RI = Pf on the resistant cultivar or rootstocks/Pf on susceptible cultivar x 100) were calculated (Ornat et al., 2001; Cortada et al., 2008). The resistance level of tomato cultivars and rootstocks carrying the *Mi-1.2* gene to each RKN isolate was categorized according to the RI as highly resistant (RI < 10%), moderately resistant ($10 \leq RI < 50\%$), or susceptible ($RI \geq 50\%$) (Cortada et al., 2009).

Data Analysis

Host reaction of tomato genotypes to each RKN isolate in the short and long growing periods was analyzed separately. Data on GI and Rf were log-transformed [log10(x+1)] prior to analysis, and then subjected to analysis of variance

(ANOVA). The Tukey HSD was used to compare means when the ANOVA analysis was significant ($p < 0.05$). Analyses were performed using SAS statistical software (SAS Institute, Cary, NC, USA).

Results

Detection of *Mi-1.2* Gene

In the amplified PCR products from DNA of all rootstocks except 'Comfort', a single band of 380 bp was obtained, indicating the homozygous resistant form (*MiMi*) at the *Mi* locus (Fig. 1). The cultivars 'Alsancak', 'Crisol', 'Esin', 'Adamset', and rootstock 'Comfort' gave two bands of 380 and 430 bp that were classified as heterozygous resistant (*Mi/mi*). 'Barbaros' used as a susceptible control displayed a single DNA fragment with 430 bp, confirming the absence of the *Mi-1.2* gene (*mi/mi*).

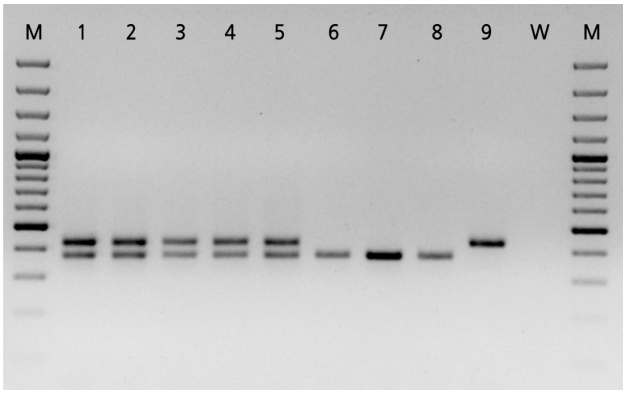

Fig. 1. Detection of the *Mi-1.2* gene in tomato cultivars (lanes 1-5, 9) and rootstocks (lanes 6-8) using primers Mi23F and Mi23R. M: Molecular marker with 100 bp, 1: 'Adamset', 2: 'Alsancak', 3: 'Crisol', 4: 'Esin', 5: 'Comfort', 6: 'Arazi', 7: 'Beaufort', 8: 'Kingkong', 9: 'Barbaros', W: water as a negative control.

Bioassay

As expected, the highest GI values in both growing periods were recorded on the susceptible control 'Barbaros', ranging from 6.0 (*M. javanica* Çr-27) to 7.0 (*M. javanica* Ço-1) in the short period experiment and from 7.4 (*M. arenaria* Sn-11 and B-17) to 8.2 (*M. incognita* Pr-4) in the long period experiment (Table 2). In the short period experiment, GI values were lower on tomato genotypes with *Mi-1.2* gene than on the susceptible control 'Barbaros' ($p < 0.05$), with the exception of the resistant 'Crisol' inoculated with *M. incognita* A-11 that had a GI of 5.4 and did not differ from the susceptible control ($p > 0.05$). Moreover, resistant tomato cultivars had higher galling rates than resistant rootstocks in the short period experiment when inoculated with *M. incognita* A-11 ($p < 0.05$). Similarly, GI values on all resistant rootstocks except 'Kingkong' were lower than on the resistant cultivars inoculated with *M. arenaria* Er-4 in this experiment ($p < 0.05$), but not in the long period experiment. There were no differences ($p > 0.05$) in galling rate among resistant cultivars for each nematode isolate when resistant genotypes were inoculated with *M. arenaria* Sn-11 or *M. incognita* Pr-4 in the short growing period and *M. arenaria* A-7, B-17 or *M. javanica* Ço-1 in the long growing period.

Table 2. Gall index (GI)[z] of *Meloidogyne arenaria* (Er-4, A-7, Sn-11, B-17), *M. javanica* (Ço-1, Çr-27), and *M. incognita* (Pr-4, A-11) on tomato cultivars and rootstocks in pot experiments conducted in a non-temperature controlled greenhouse for short (8 weeks) and long (16 weeks) growing periods after inoculation with 3,000 eggs per plant

Tomato	*M. arenaria*				*M. javanica*		*M. incognita*	
	Er-4	A-7	Sn-11	B-17	Ço-1	Çr-27	Pr-4	A-11
Short period								
Rootstock								
Arazi	0.20 ± 0.20[y]c[x]	0.20 ± 0.20 c	0.40 ± 0.24 b	0.20 ± 0.20 c	1.20 ± 0.20 bc	0.60 ± 0.24 c	0.40 ± 0.24 b	1.40 ± 0.24 d
Beaufort	0.20 ± 0.20 c	0.60 ± 0.24 bc	0.40 ± 0.24 b	0.60 ± 0.24 bc	0.40 ± 0.24 c	1.40 ± 0.24 bc	0.80 ± 0.37 b	0.60 ± 0.24 e
Comfort	0.20 ± 0.20 c	0.20 ± 0.20 c	0.40 ± 0.24 b	0.20 ± 0.20 c	0.60 ± 0.24 bc	1.20 ± 0.20 bc	1.00 ± 0.00 b	1.00 ± 0.00 de
Kingkong	0.60 ± 0.24 bc	0.60 ± 0.24 bc	0.40 ± 0.24 b	0.40 ± 0.24 bc	0.40 ± 0.24 c	1.40 ± 0.24 bc	0.80 ± 0.37 b	1.40 ± 0.24 d
Cultivar								
Adamset	1.00 ± 0.00 b	1.00 ± 0.31 bc	1.40 ± 0.24 b	0.80 ± 0.20 bc	1.40 ± 0.24 bc	2.00 ± 0.31 b	0.60 ± 0.24 b	4.20 ± 0.20 bc
Alsancak	1.40 ± 0.24 b	1.20 ± 0.20 b	1.20 ± 0.20 b	0.20 ± 0.20 c	0.40 ± 0.24 c	0.40 ± 0.24 c	0.40 ± 0.24 b	2.80 ± 0.20 c
Crisol	1.40 ± 0.24 b	1.60 ± 0.24 b	1.40 ± 0.24 b	1.60 ± 0.40 b	1.60 ± 0.24 b	2.40 ± 0.24 b	1.00 ± 0.00 b	5.40 ± 0.24 ab
Esin	1.00 ± 0.00 b	0.80 ± 0.20 bc	0.80 ± 0.37 b	0.20 ± 0.20 c	0.40 ± 0.24 c	1.40 ± 0.24 bc	1.40 ± 0.24 b	3.60 ± 0.24 bc
Barbaros	6.60 ± 0.24 a	6.40 ± 0.40 a	6.40 ± 0.24 a	6.40 ± 0.40 a	7.00 ± 0.31 a	6.00 ± 0.31 a	6.60 ± 0.24 a	6.40 ± 0.24 a
Long period								
Rootstock								
Arazi	2.40 ± 0.24 bc	1.40 ± 0.24 b	1.40 ± 0.24 c	1.60 ± 0.24 b	2.40 ± 0.24 b	1.80 ± 0.20 e	2.40 ± 0.24 d	5.00 ± 0.00 c
Beaufort	2.20 ± 0.37 bc	2.40 ± 0.50 b	1.80 ± 0.20 bc	2.00 ± 0.31 b	2.00 ± 0.00 b	3.20 ± 0.37 cd	3.00 ± 0.31 cd	1.60 ± 0.24 d
Comfort	1.60 ± 0.24 c	1.60 ± 0.24 b	2.00 ± 0.31 bc	1.80 ± 0.20 b	2.00 ± 0.31 b	2.40 ± 0.24 cd	3.80 ± 0.37 bc	4.40 ± 0.24 c
Kingkong	3.00 ± 0.31 b	2.20 ± 0.48 b	2.00 ± 0.00 bc	1.80 ± 0.20 b	1.40 ± 0.24 b	3.40 ± 0.40 cd	3.40 ± 0.24 bcd	4.80 ± 0.20 c
Cultivar								
Adamset	1.80 ± 0.20 bc	2.20 ± 0.37 b	2.40 ± 0.40 bc	1.40 ± 0.24 b	2.40 ± 0.24 b	4.20 ± 0.37 bc	2.60 ± 0.24 cd	5.20 ± 0.20 c
Alsancak	2.20 ± 0.20 bc	2.20 ± 0.20 b	2.40 ± 0.24 bc	1.20 ± 0.20 b	1.40 ± 0.24 b	1.40 ± 0.24 e	2.60 ± 0.24 cd	5.40 ± 0.24 c
Crisol	2.40 ± 0.24 bc	2.60 ± 0.24 b	2.80 ± 0.37 b	2.40 ± 0.50 b	2.20 ± 0.20 b	4.60 ± 0.24 b	3.00 ± 0.31 cd	6.80 ± 0.37 b
Esin	2.20 ± 0.20 bc	1.80 ± 0.20 b	1.80 ± 0.20 bc	1.20 ± 0.20 b	1.40 ± 0.24 b	2.20 ± 0.20 de	4.40 ± 0.24 b	5.40 ± 0.24 c
Barbaros	7.80 ± 0.37 a	7.80 ± 0.37 a	7.40 ± 0.24 a	7.40 ± 0.24 a	7.60 ± 0.24 a	8.00 ± 0.31 a	8.20 ± 0.37 a	8.00 ± 0.31 a

[z]GI was based on a scale from 0 (none) to 10 (dead plants).

[y]Values were transformed [log10(x + 1)] before analysis. Data represent mean of five replicates ± standard errors. Each growing period was subjected separately to analysis for each nematode isolate.

[x]Values in the same column sharing the same letter are not significantly different according to Tukey's HSD test at $p < 0.05$.

Similar to galling rates, the highest reproduction rates of nematode isolates were detected on the susceptible 'Barbaros' ($p < 0.05$) (Table 3). In the short period experiment, only *M. incognita* A-11 had higher Pf values than Pi on the resistant cultivars with Rf ranging from 1.59 to 2.46 and these values on the resistant cultivars were significantly higher than on the resistant rootstocks ($p < 0.05$). Similarly, irrespective of the statistical significance, egg productions of this nematode isolate on the resistant cultivars were higher than on the rootstocks in the long period experiment. For all nematode isolate-plant genotype combinations, Rf values on resistant cultivars and rootstocks were similar when inoculated with *M. arenaria* Er-4 or *M. incognita* Pr-4 for short period and *M. arenaria* Sn-11 for the long period ($p > 0.05$). Although Pf value of *M. javanica* Çr-27 on resistant cultivar Crisol was close to Pi value in the short period experiment, it was 5.8-fold higher than Pi in the long period experiment. In the long period experiment, of the resistant cultivars and rootstocks

inoculated with *M. javanica* Çr-27 and *M. incognita* A-11, 'Crisol' supported more nematode reproduction than the others, whereas 'Esin' resulted in the highest Rf of *M. incognita* Pr-4 ($p < 0.05$). The lowest Rf value of *M. incognita* A-11 in the long period experiment was detected on the rootstock Beaufort, with a Rf of 0.5 ($p < 0.05$), whereas Rf values of the isolate on other resistant genotypes ranged from 2.82 to 11.09.

All cultivars and rootstocks carrying the *Mi-1.2* gene in both growing periods responded highly resistant (RI < 10%) to RKN isolates tested except for *M. incognita* A-11 (Fig. 2). Of the all resistant genotypes inoculated with *M. incognita* A-11, in both growing periods, 'Crisol' (RI = 12.01% and 26.11%) and 'Adamset' (RI = 10.86% and 16.11%) were moderately resistant, whereas 'Beaufort' (RI = 0.38% and 1.17%) and 'Comfort' (RI = 0.44% and 6.57%) were highly resistant. Although 'Alsancak' (RI = 7.77%), 'Esin' (RI = 7.89%), 'Arazi' (RI = 0.91%), and 'Kingkong' (RI = 0.82%) were highly resistant to *M. incognita* A-11 in the short growing period, these genotypes showed reduced resistance and responded as moderately resistant to the nematode isolate in the long growing period.

Table 3. Reproduction rate (Rf)[z] of *Meloidogyne arenaria* (Er-4, A-7, Sn-11, B-17), *M. javanica* (Ço-1, Çr-27), and *M. incognita* (Pr-4, A-11) on tomato cultivars and rootstocks in pot experiments conducted in a non-temperature controlled greenhouse for short (8 weeks) and long (16 weeks) growing periods after inoculation with 3,000 eggs per plant

Tomato	*M. arenaria*				*M. javanica*		*M. incognita*	
	Er-4	A-7	Sn-11	B-17	Ço-1	Çr-27	Pr-4	A-11
Short period								
Rootstock								
Arazi	0.04 ± 0.04[y]b[x]	0.04 ± 0.04 bc	0.03 ± 0.02 c	0.01 ± 0.01 c	0.22 ± 0.06 bc	0.04 ± 0.01 d	0.09 ± 0.06 b	0.18 ± 0.04 d
Beaufort	0.04 ± 0.04 b	0.11 ± 0.04 bc	0.05 ± 0.04 c	0.07 ± 0.03 c	0.06 ± 0.04 c	0.34 ± 0.08 cd	0.14 ± 0.06 b	0.07 ± 0.03 d
Comfort	0.03 ± 0.03 b	0.02 ± 0.02 c	0.07 ± 0.04 c	0.02 ± 0.02 c	0.12 ± 0.05 bc	0.28 ± 0.02 cd	0.21 ± 0.04 b	0.09 ± 0.00 d
Kingkong	0.17 ± 0.08 b	0.10 ± 0.04 bc	0.02 ± 0.01 c	0.06 ± 0.03 c	0.04 ± 0.03 c	0.37 ± 0.11 cd	0.17 ± 0.09 b	0.16 ± 0.03 d
Cultivar								
Adamset	0.13 ± 0.03 b	0.27 ± 0.07 bc	0.33 ± 0.13 bc	0.12 ± 0.03 c	0.33 ± 0.07 bc	0.83 ± 0.17 bc	0.08 ± 0.02 b	2.22 ± 0.34 bc
Alsancak	0.15 ± 0.02 b	0.37 ± 0.06 bc	0.28 ± 0.06 bc	0.02 ± 0.02 c	0.03 ± 0.02 c	0.03 ± 0.02 d	0.04 ± 0.02 b	1.59 ± 0.13 c
Crisol	0.41 ± 0.11 b	0.55 ± 0.15 b	0.49 ± 0.15 b	0.68 ± 0.16 b	0.44 ± 0.11 b	1.03 ± 0.10 b	0.18 ± 0.04 b	2.46 ± 0.13 b
Esin	0.10 ± 0.00 b	0.09 ± 0.06 bc	0.18 ± 0.07 bc	0.01 ± 0.01 c	0.08 ± 0.05 c	0.27 ± 0.07 cd	0.44 ± 0.10 b	1.61 ± 0.14 c
Barbaros	39.20 ± 5.96 a	31.60 ± 5.27 a	43.60 ± 4.11 a	26.20 ± 4.61 a	40.40 ± 4.36 a	29.20 ± 4.38 a	33.60 ± 5.17 a	20.50 ± 1.77 a
Long period								
Rootstock								
Arazi	1.05 ± 0.09 b	0.26 ± 0.06 d	0.40 ± 0.06 b	0.42 ± 0.10 bcd	1.19 ± 0.21 b	0.62 ± 0.09 ef	0.92 ± 0.10 d	5.13 ± 0.40 cd
Beaufort	0.92 ± 0.09 b	0.89 ± 0.28 bcd	0.51 ± 0.10 b	0.66 ± 0.13 bc	0.52 ± 0.06 bc	1.24 ± 0.21 de	1.10 ± 0.23 cd	0.50 ± 0.11 f
Comfort	0.31 ± 0.08 d	0.29 ± 0.07cd	0.61 ± 0.08 b	0.37 ± 0.07 bcd	0.59 ± 0.12 bc	1.02 ± 0.07 de	1.82 ± 0.22 c	2.82 ± 0.28 e
Kingkong	1.28 ± 0.21 b	0.63 ± 0.18 bcd	0.78 ± 0.06 b	0.70 ± 0.11 b	0.44 ± 0.10 c	1.75 ± 0.30 d	1.15 ± 0.05 cd	4.51 ± 0.54 d
Cultivar								
Adamset	0.48 ± 0.07 cd	0.72 ± 0.16 bcd	1.03 ± 0.38 b	0.35 ± 0.06 bcd	1.20 ± 0.22 b	3.28 ± 0.34 c	0.97 ± 0.13 d	6.93 ± 0.18 c
Alsancak	0.38 ± 0.05 cd	0.92 ± 0.13 bc	1.11 ± 0.19 b	0.18 ± 0.01 d	0.42 ± 0.13 c	0.38 ± 0.10 f	0.89 ± 0.09 d	6.56 ± 0.26 cd
Crisol	0.76 ± 0.05 bc	1.24 ± 0.12 b	1.06 ± 0.13 b	0.81 ± 0.20 b	0.88 ± 0.13 bc	5.83 ± 0.58 b	1.26 ± 0.14 cd	11.09 ± 0.88 b
Esin	0.38 ± 0.07 cd	0.42 ± 0.09 cd	0.53 ± 0.12 b	0.23 ± 0.03 cd	0.44 ± 0.16 c	0.95 ± 0.15 de	3.24 ± 0.30 b	7.21 ± 0.77 c
Barbaros	54.74 ± 2.18 a	57.26 ± 2.97 a	67.61 ± 4.37 a	49.98 ± 2.90 a	62.17 ± 3.76 a	61.11 ± 5.42 a	61.58 ± 3.93 a	43.04 ± 3.55 a

[z]Rf = Final population density / Initial population density.

[y]Values were transformed [log10(x + 1)] before analysis. Data represent mean of five replicates ± standard errors. Each growing period was subjected separately to analysis for each nematode isolate.

[x]Values in the same column sharing the same letter are not significantly different according to Tukey's HSD test at $p < 0.05$.

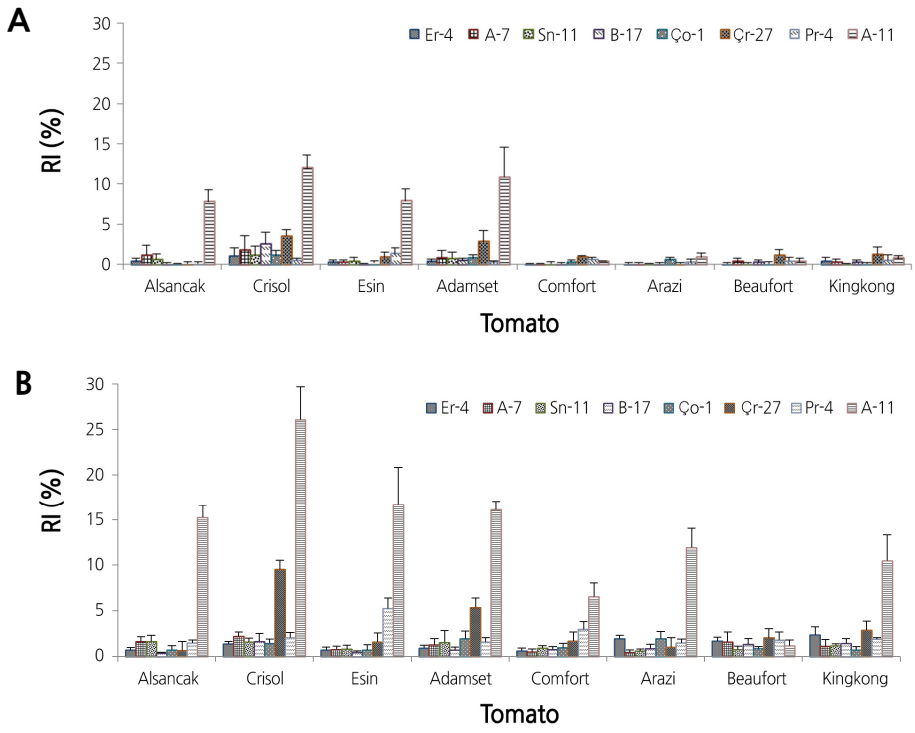

Fig. 2. Reproduction index (RI) of *Meloidogyne arenaria* (Er-4, A-7, Sn-11, B-17), *M. javanica* (Ço-1, Çr-27), and *M. incognita* (Pr-4, A-11) on resistant tomato cultivars ('Alsancak', 'Crisol', 'Esin', and 'Adamset') and rootstocks ('Comfort', 'Arazi', 'Beaufort', and 'Kingkong') in pot experiments conducted in a non-temperature controlled greenhouse for (A) short (8 weeks) and (B) long (16 weeks) growing periods after inoculation with 3,000 eggs per plant. RI: eggs per plant on a resistant tomato divided by eggs per plant on susceptible control×100.

Discussion

Several molecular markers were developed to detect the presence of the *Mi-1.2* gene in tomato, which confers resistance to *M. arenaria*, *M. javanica*, and *M. incognita* (Williamson et al., 1994; El Mehrach et al., 2005; Seah et al., 2007). Molecular markers successfully used in tomato breeding also enable determination of whether the *Mi-1.2* gene is homozygous or heterozygous (Cortada et al., 2008; Devran et al., 2013). Of the molecular markers related to the *Mi-1.2* gene, REX-1 has been widely used but some studies revealed that this marker gave false positive results in plants carrying the *Ty-1* gene, which provides resistance to tomato yellow leaf curl virus (TYLCV) because both the *Mi-1.2* gene and the *Ty-1* gene are located on chromosome 6 and are very close to each other (El Mehrach et al., 2005; Seah et al., 2007; Devran et al., 2013). Therefore, the marker REX-1 could not be used for screening of the *Mi-1.2* gene in tomato plants carrying the *Ty-1* gene (El Mehrach et al., 2005; Devran et al., 2013). Moreover, the amplified products of the REX-1 marker have to be digested with a restriction enzyme (*Taq*I) (Williamson et al., 1994). Alternatively, PMi12 and Mi23 markers can be used, which are reliable markers, require no restriction digestion, and allow differentiation between homozygous and heterozygous resistant genotypes (El Mehrach et al., 2005; Seah et al., 2007; Devran et al., 2013). In this study, we preferred to use the Mi23 marker because the PMi12 marker produced additional bands in some of the tomato lines tested by Devran et al. (2013). Our Mi23 marker analysis was in accordance with previous reports regarding the resistant genotypes 'Beaufort' (Cortada et al., 2008), 'Alsancak', and 'Esin' (Devran et al., 2013).

Seventy-two nematode isolate-plant genotype combinations were tested in both short and long period experiments to evaluate the resistance level of tomato cultivars and rootstocks against different RKN isolates. RI values on resistant genotypes were higher in the long growing period than the short growing period, irrespective of the statistical significance. A likely reason for the differential RI values on the resistant genotype between the two experimental periods is the temperature since the experiments were performed in a greenhouse with a non-controlled temperature environment. During the long period experiment, elevated air temperatures above 28°C were registered, providing conditions that could break the resistance mediated by the *Mi-1.2* gene (Dropkin, 1969; Ammati et al., 1986; Devran et al., 2010). Verdejo-Lucas et al. (2013) stated that phenotypic expression of the *Mi-1.2* gene was not compromised under daily temperature fluctuations and intermittent peaks above 28°C, and the stability of the *Mi-1.2* gene was affected by heat intensity, the length of the heated period, or their interaction. A constant temperature of 32°C for 2-3 days was at least required to overcome the resistance (Dropkin, 1969; Araujo et al., 1982b). In this study, none of the resistant genotypes displayed susceptible response when inoculated with any nematode isolates. Moreover, the highly resistant response of the plant genotypes carrying the *Mi-1.2* gene did not change, with the exception of those inoculated with *M. incognita* A-11, despite the observation that the RI values of these genotypes were higher depending on the experimental periods. Thus, in this study, the nematode population is more effective than the temperature in terms of influencing the category of nematode resistance in the tested cultivars and rootstocks. Of the tested nematode isolates, only *M. incognita* A-11 caused changes in the relative levels of resistance in all plant genotypes carrying the *Mi-1.2* gene, except rootstocks 'Beaufort' and 'Comfort', in the long growing period and 'Alsancak', 'Esin', 'Arazi', and 'Kingkong' exhibited reduced resistance, responding as moderately resistant. The reduced resistance response of these genotypes to this nematode isolate may be based on the existence of nematodes with different degrees of virulence within the population used for inoculation (Molinari and Caradonna, 2003; Castagnone-Sereno et al., 2007). Previously, *M. incognita* A-11, unlike the other nematode isolates tested in the present study, showed RI higher than 10% (RI=15.6), indicating partial virulence of the isolate (Aydınlı, 2014). Therefore, the differential resistance levels of some genotypes against nematode isolates are likely related to nematode virulence. Even if virulent individuals in Pi were present at a low frequency, the rates of virulence will gradually increase during a prolonged growing period of plants. In contrast, the rootstocks 'Beaufort' and 'Comfort' were classified as highly resistant against *M. incognita* A-11 in the long growing period. This could be due to the plant genetic background, which might contribute to the stability of *Mi-1.2* resistance in the presence of a partially virulent population. On the other hand, the rootstock 'Beaufort' was classified as susceptible to *M. incognita* (Lopez-Perez et al., 2006) and *M. javanica* (Cortada et al., 2008, 2009) in previous studies. The response of 'Beaufort' to a *Mi*-avirulent population of *M. javanica* was susceptible after both one (63 days post-inoculation) and two nematode generations (132 days post-inoculation) (Cortada et al., 2008). However, Cortada et al. (2009) reported that the resistance level of 'Beaufort' varied depending on the *Meloidogyne* isolates, and the response of the rootstock resulted in classification as highly resistant to *M. arenaria* (MA-68) and *M. incognita* (MI-CROS), moderately resistant to *M. incognita* (MI-ALM), and susceptible to two *M. javanica* (MJ-IBIZA and MJ-05). Additionally, the rootstock 'Beaufort' displayed high resistance to *M. incognita* race 2 at constant 24°C, but moderate resistance at constant 32°C (Devran et al., 2010). Our study indicated that rootstocks 'Beaufort' and 'Comfort' could be used as an alternative to other resistant genotypes tested in this study if the emergence of virulent nematode populations in infested fields occurs.

It is well documented that the resistance provided by the *Mi-1.2* gene could be affected by nematode population (Ornat

et al., 2001; Cortada et al., 2008, 2009), plant genetic background (Tzortzakakis et al., 1998; Jacquet et al., 2005; Lopez-Perez et al., 2006; Verdejo-Lucas et al., 2009), and temperature (Dropkin, 1969; Araujo et al., 1982a, 1982b; Ammati et al., 1986; Devran et al., 2010; Verdejo-Lucas et al., 2013; Carvalho et al., 2015). However, there were only limited studies on the resistance response of tomato cultivars and rootstocks with the *Mi-1.2* gene to different isolates of RKN depending on the duration of the experiment (Cortada et al., 2008, 2009). The present study confirms the previous studies and reveals that the relative resistance levels of cultivars and rootstocks could vary according to RKN isolates or the length of the growing period. The differential response of the resistant genotype to *Meloidogyne* isolates may arise from the proportion of virulent individual nematodes within a population (Molinari and Caradonna, 2003; Castagnone-Serona et al., 2007). Therefore, the characteristics of the nematode population should be taken into account in the choice of tomato cultivars and rootstocks for site-specific management of RKN. Moreover, in order to determine the suitable resistant genotype for the growing area where there may be a virulent population, it may be necessary to assess a long growing period, which allows the detection of population increases and changes in the relative levels of resistance in plant genotypes.

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