Horticultural Science/ Original Article

Yield and resistance of tomato rootstocks to Meloidogyne arenaria in a greenhouse

Abstract – The objective of this work was to evaluate tomato rootstocks with the Mi resistance gene for nematode control and fruit yield, in a greenhouse naturally infested with Meloidogyne arenaria. The experiment was carried out in a randomized complete block design with four treatments (three resistant rootstocks and the Barbaros susceptible cultivar) and four replicates per treatment. 'Barbaros' was grafted onto the 'Arazi', 'Beauford', and 'King Kong' rootstocks, and compared with nongrafted 'Barbaros'. The maximum daily ambient temperatures in the greenhouse was above 28°C, in some days, during the growing period. The grafted tomato had a lower galling index and egg production than the susceptible tomato; however, no significant differences were observed between the three rootstocks. Likewise, there was no difference for the total cumulative fruit yield between rootstocks. All grafted tomatoes also had significantly higher fruit yields than the nongrafted control. The rootstocks provided fruit yields from 40.07% to 63.86% higher than that of the susceptible control in the nematode-infested soil. The rootstocks with the Mi resistance gene favor a higher tomato fruit yields and inhibit the nematode increase in soils infested with M. arenaria.

Index terms: Solanum lycopersicum, control, reproduction, root-knot nematode.

Rendimento e resistência de porta-enxertos de tomate a Meloidogyne arenaria em cultivo em estufa

Resumo – O objetivo deste trabalho foi avaliar porta-enxertos de tomate com o gene de resistência Mi quanto ao controle de nematoídes e à produção de frutos, em casa de vegetação naturalmente infestada com Meloidogyne arenaria. O experimento foi realizado em delineamento de blocos ao acaso, com quatro tratamentos (três porta-enxertos resistentes e a cultivar suscetível Barbaros) e quatro repetições por tratamento. A cultivar 'Barbaros' foi enxertada nos porta-enxertos 'Arazi', 'Beauford' e 'King Kong' e comparada com a 'Barbaros' não enxertada. A temperatura ambiente máxima diária, na estufa, foi superior a 28 °C, em alguns dias, durante o período de crescimento. Os tomates enxertados apresentaram menor índice de galhas e produção de ovos do que os não enxertados; no entanto, não se observaram diferenças significativas entre os três porta-enxertos. Semelhantemente, não houve diferença quanto à produção cumulativa total de frutos entre os porta-enxertos. Os tomates enxertados também apresentaram produtividade de frutos significativamente maior do que o não enxertado. Os porta-enxertos proporcionaram rendimentos de frutos de 40,07% a 63,86% maiores do que os da cultivar suscetível no solo infestado com nematoídes. Os porta-enxertos com o gene de resistência Mi favorecem a maior produtividade do tomate e inibem o aumento de nematoídes em solos infestados com M. arenaria.

Termos para indexação: Solanum lycopersicum, controle, reprodução, nematoide-das-galhas.
Introduction

Root-knot nematodes (Meloidogyne spp.) are one of the most serious pests of tomato (Solanum lycopersicum) and cause crop damage and yield reduction, especially in greenhouses where favorable environmental conditions support high nematode reproduction when susceptible crops are present. Damages depend on the nematode species, initial population density, cultivated crop species, and a range of environmental factors; tomato yield losses have been reported, and they may reach up to 100% (Seid et al., 2015). More than 100 species of the genus Meloidogyne have been described, and M. arenaria, M. incognita, and M. javanica are cited as major species because of their wide distribution and economic importance (Elling, 2013). Although these major species infest tomato greenhouses in many regions of the world, M. incognita and M. javanica are more widespread than M. arenaria in the Mediterranean region, including the south of Turkey (Karajeh et al., 2005; Tzortzakakis et al., 2005; Devran & Söğüt, 2009; Talavera et al., 2012; Uysal et al., 2017; Janati et al., 2018). In contrast, M. arenaria is the most common root-knot nematode detected in greenhouses of Black Sea Region, in the north of Turkey (Aydınlı & Mennan, 2016).

The control of root-knot nematodes in greenhouse has been conventionally relied upon chemical treatment. Reducing the use of synthetic nematicides and offering alternative strategies for nematode control is key in sustainable agriculture systems. Host plant resistance has been considered as an attractive alternative because nematode control is achieved with little cost to the farmers, without harmful effects on human and environment health. Additionally, there is no need for any skill in application methods (Verdejo-Lucas & Sorribas, 2008). Due to these advantages, resistance is one of the major components of integrated nematode management (Seid et al., 2015). In tomato, the resistance against the above-mentioned major Meloidogyne species is mediated by the single dominant Mi-1.2 gene that was introgressed from a wild tomato species, Solanum peruvianum. The Mi-1.2-mediated resistance is associated with a localized hypersensitive response that is characterized by plant cell death, near or at the feeding site, of the second-stage juvenile nematodes, possibly resulting in nematode death because of starvation (Verdejo-Lucas et al., 2012). Therefore, cultivars with Mi-1.2 gene can be grown in nematode-infested soil, without significant yield losses, and they could provide benefits to the subsequent crop because of the reduced nematode population density in the soil (Talavera et al., 2009).

Despite the resistant tomato cultivars available for greenhouse production, farmers in the Black Sea region of Turkey have preferred to grow susceptible cultivars adapted to the prevailing climatic conditions in the growing period, and which matched with the market standards and consumer preferences for characteristics such as shape, size, and flavor. However, the cultivation of susceptible cultivars in nematode-infested greenhouses may result in significant yield losses, when chemical approaches are not used. Thus, the grafting of these cultivars onto nematode-resistant rootstocks is one of the best eco-friendly solutions to avoid damage by root-knot nematodes. Rootstocks can also offer multiple pathogen resistance and protection from abiotic factors, such as salinity, water stress, and low and high temperature stresses (Cortada et al., 2008; Devran et al., 2010; Singh et al., 2017; Grieiesen et al., 2018).

Previous studies conducted in different regions of the world showed that tomato rootstocks carrying Mi gene were effective for the control of Meloidogyne (Lopez-Perez et al., 2006; Cortada et al., 2008, 2009; Verdejo-Lucas et al., 2009, 2013; Devran et al., 2010; Rivard et al., 2010; Owusu et al., 2016; Aydınlı & Mennan, 2019). However, several studies reported that variable reproduction rates of the same Meloidogyne isolate among resistance rootstocks were detected, resulting in differential resistance response (Lopez-Perez et al., 2006; Cortada et al., 2008, 2009; Rivard et al., 2010; Verdejo-Lucas et al., 2013; Aydınlı & Mennan, 2019). According to these results, the response of tomato rootstocks against root-knot nematodes varies greatly, depending on the plant genotype. Comparatively, some studies have addressed the effect of rootstocks on tomato yield, in naturally infested soil with the root-knot nematode M. incognita and M. javanica (Verdejo-Lucas & Sorribas, 2008; Kaşkavalcı et al., 2009; Verdejo-Lucas et al., 2009; Rivard et al., 2010; Duran Akkurt et al., 2013; Owusu et al., 2016). However, none of tomato rootstocks have been tested for yield performance and resistance against M. arenaria.
The objective of this work was to address rootstocks with the \( Mi \) resistance gene for nematode control and tomato fruit yield, in a greenhouse naturally infested with \textit{Meloidogyne arenaria}.

**Materials and Methods**

The experiment was carried out during a summer-growing season (1 May – 19 September 2014), in a greenhouse naturally infested with \textit{M. arenaria} located in the experimental station of the Faculty of Agriculture, Ondokuz Mayis University (Samsun Province, Turkey). A randomized complete block design was used with four plant treatments (three tomato rootstocks, and a susceptible tomato cultivar), and four replicates per treatment. Each treatment represented a plot with 10 tomato plants. The treatments were three ‘Barbaros’ graftings and a control, as follows: ‘Barbaros’ (Seven Brothers Seeds) grafted onto the resistant tomato rootstock ‘Arazi’ (Sygenta Seeds); ‘Barbaros’ grafted onto the resistant tomato rootstock ‘Beaufort’ (De Ruiter Seeds); ‘Barbaros’ grafted onto the resistant tomato rootstock ‘King Kong’ (Rijk Zwaan Seeds); and the control, nongrafted ‘Barbaros’.

The greenhouse (120 m\(^2\)) was unheated, covered with polyethylene plastic, and had a sandy soil with 7.9 pH, 0.31 dS m\(^{-1}\) electrical conductivity, 2.35% organic matter, and 2.32% CaCO\(_3\). Before the experiment, cucumber was cultivated in this greenhouse, in the 2013 summer-growing season, and heavily galled roots of cucumber were observed at the end of that period. To maintain the high natural nematode population in the soil, roots of cucumber were not removed from it, and the greenhouse remained without cultivation until this experiment.

The soil was plowed to 30 cm depth, using a garden rotavator, on 9 April 2014. In the previous cultivation system in the greenhouse, beds were shaped, and individual plots were marked (0.7 × 3.0 m) on the beds. Sixteen plots were designed (on 29 April 2014), and four soil samples were collected from the rhizosphere zone (at 5-30 cm soil depth), in a zigzag pattern within each plot, and mixed thoroughly to represent a composite sample per plot. After the soil sampling, a drip irrigation system was placed, and the beds were covered with black polyethylene mulch. Individual plots consisted of two rows, with five plants of tomato seedling per row. Seedlings were planted on 1 May 2014, at 50 cm within the row, and 50 cm between rows, in each plot. The grafted and nongrafted tomato seedlings were purchased from a commercial nursery (Olympus Fide, Antalya, Turkey). The homozygous resistant for the \( Mi \) locus (\( Mi/Mi \)) in rootstocks and the absence of the \( Mi-1.2 \) gene (\( mi/mi \)) in susceptible tomato cultivar were verified using the molecular marker Mi23 in our previous study (Aydınlı & Mennan, 2019).

Soil samples collected from plots were used to determine the initial nematode population density (Pi), and to confirm the presence of \textit{M. arenaria} in the greenhouse. Three of 100 cm\(^3\) individual subsamples from each composite sample were used for nematode extraction by tray method (Whitehead & Hemming, 1965). Every two days, nematode suspension was collected from trays, and then fresh water was added. Trays were left until the absence of nematode, and extracted nematodes of respective 100 cm\(^3\) subsamples were pooled. Second-stage juveniles (J2) of \textit{Meloidogyne} were counted and expressed per 100 cm\(^3\) of soil. Average initial nematode populations in greenhouse were \( 365±174 \) (mean±standard deviation) 100 cm\(^3\) soil, and the average infestation levels of plant treatments were similar (Table 1). After nematode extraction, the remaining soil of the composite sample collected from each plot was transferred to pots, and a single four-week-old seedling of nematode-susceptible tomato cultivar Falcon was transplanted into each pot. Forty-five days after transplanting, individual females from roots were extracted to perform their esterase phenotypes and, thus, the presence of \textit{M. arenaria} in each plot was confirmed.

Daily ambient temperatures in the greenhouse, including maximum (highest) and minimum (lowest) ones, were recorded once every day, during the experiment period. The mean daily ambient temperatures ranged from 16 to 36°C in the experiment (mean = 25.8°C) (Figure 1). Tomato fruit were harvested in the first time on 10 July, and fruit weight per plot was recorded until the experiment finished on 19 September; the cumulative yield was expressed (kg m\(^{-2}\)) (Verdejo-Lucas & Sorribas, 2008). After the final fruit harvest, the plants were cut at ground level, and roots were removed from the soil, washed, and rated for galling index using a 0–10 scale (Bridge & Page, 1980). Then, the root system of four plants from each plot was immersed in Phlokosin B solution (15 mg L\(^{-1}\)) for 20 min.
to stain the egg masses, and the total egg masses per root system was counted (Hartman & Sasser, 1985). Then, roots from each plot were bulked, cut into 1–2 cm pieces, and four 10 g subsamples of roots were used to extract eggs by blender maceration in a 1% NaOCl solution (Hussey & Barker, 1973). The number of eggs was expressed (g\(^{-1}\) root). The resistance of a plant genotype is evaluated for root-knot nematode based on the suppressive nematode reproduction and is related to reproduction on a susceptible plant host (Cortada et al., 2008). Thus, the reproduction index (RI) was used to detect the resistance levels of tomato genotypes tested for root-knot nematodes, and plant response was categorized as highly resistant (RI<10%), moderately resistant (10≤RI<50%), or susceptible (RI≥50%) (Cortada et al., 2008, 2009). The RI of nematodes on the rootstocks was calculated as the number of eggs per g root on the resistant rootstock, divided by the number of eggs per g root on the susceptible cultivar Barbaros × 100 (Verdejo-Lucas et al., 2009).

Results and Discussion

The tomatoes grafted on Mi rootstocks showed lower galling index and egg production than the susceptible cultivar (Table 1). Mean root galling indices for the rootstocks ranged from 0.95 ('Beaufort') to 2.0 ('King Kong'); however, significant differences between rootstocks were not observed. Some plants of the rootstocks had no root gall, whereas all plants of the susceptible cultivar showed galling on their roots. The number of egg masses on the susceptible cultivar was approximately 22, 27, and 38 times higher than on the rootstocks 'King Kong', 'Arazi', and 'Beaufort', respectively. The lowest numbers of eggs per root were recorded on the rootstock 'Beaufort', but were not different from values on other rootstocks. The RI of nematodes was similar for rootstocks, although nearly two times more RI were recorded on 'Arazi' than on 'Beaufort'.

The tomato rootstocks carrying the Mi-1.2 gene showed remarkable suppressive effects on M. arenaria for nematode reproduction and nematode damage (root galling) on their roots in comparison with the nongrafted susceptible tomato cultivars (Table 1). The relative resistance level of rootstocks varied according to RI, although there were no significant differences on RI, the values ranged from 9.78% (Beaufort) to 18.08% (Arazi). 'King Kong' and 'Arazi' exhibited lower resistance levels than 'Beaufort' that responded as highly resistant to M. arenaria. In a pot experiment conducted in a plastic greenhouse naturally infested with Meloidogyne arenaria.
Yield and resistance of tomato rootstocks to *Meloidogyne arenaria*

...resistance to *M. arenaria,* *M. incognita,* and *M. javanica,* except for a population of *M. incognita* (A-11) in the long growing period (Aydinli & Mennan, 2019). However, the results obtained on resistance levels of rootstocks against *M. incognita* (A-11), for the long growing period, was a similar trend to that of the present study, indicating a higher nematode resistance level of 'Beaufort' than that of 'King Kong' and 'Arazi'. Another study from Turkey reported that 'Beaufort' was highly resistant to *M. incognita* at constant 24°C, but it showed a reduced resistance at constant 32°C and, thus, the resistance-breaking was attributed to high temperature (Devran et al., 2010). Nematode resistance mediated by the Mi-1.2 gene breaks at temperatures above 28°C (Dropkin, 1969). However, a highly resistance level (RI=10%) to *M. arenaria* and *M. javanica* was reported in six of eight cultivars, and in rootstocks with the Mi gene, under daily soil temperature fluctuations and intermittent peaks above 28°C, even if heating periods (hours of soil temperature above 28°C) occurred for 7.5 hours per day (Verdejo-Lucas et al., 2013). Similarly, the resistance level of the cultivar Monika to *M. javanica* was not affected under field conditions, despite the increasing nematode reproduction with the increase in the number of days under soil temperature above 28°C (Talavera et al., 2009).

The maintenance of resistance under intermittent elevated soil temperatures above 28°C could be explained by the recovery hypothesis suggested by Carvalho et al. (2015), who showed that Mi-1.2 resistance can recover with time, regardless of additional heat exposures and nematode infection. In the present study, maximum daily ambient temperatures were above 28°C in many days, during the experiment, but data on the duration of the heating period within a day was not registered. The resistance of 'Beaufort' to *M. arenaria* indicated that high temperature values in the experiment period did not compromise the resistance provided by the Mi-1.2 gene, thus confirming previous reports (Verdejo-Lucas et al., 2013; Carvalho et al., 2015).

Studies on the resistant response of rootstock 'Beaufort' to major root-knot nematode species from other countries showed variable results, although the temperature remained below 28°C (Lopez-Perez et al., 2006; Cortada et al., 2008, 2009). Lopez-Perez et al. (2006) reported the high population densities of the three *M. incognita* populations on this rootstock, in pot tests, indicating a susceptible response to this nematode species. The similar susceptible response was reported for *M. javanica* under both pot and field conditions (Cortada et al., 2008). Cortada et al. (2009) showed a differential resistance response of this rootstock containing a high resistance to *M. arenaria* MA-68 and *M. incognita* MICROs, a moderate resistance to *M. incognita* MIALM, and a susceptible resistance to *M. javanica* MJ-IBIZA and MJ-05; these authors concluded that the resistance responses of tomato rootstocks could vary according to nematode populations. This factor that caused variation in the categorization of the resistance in a plant was confirmed in our previous study (Aydinli & Mennan, 2019). 'Alsancak', 'Esin','Arazi', and 'King Kong' showed a reduced resistance and responded as moderately...

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Pi</th>
<th>GF</th>
<th>Egg mass per plant</th>
<th>Eggs g⁻¹ root</th>
<th>RI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arazi</td>
<td>459±144a</td>
<td>1.15±0.76b</td>
<td>13.50±13.29b</td>
<td>779.50±934.27b</td>
<td>18.08±21.67a</td>
</tr>
<tr>
<td>Beaufort</td>
<td>383±194a</td>
<td>0.95±0.83b</td>
<td>10.50±8.82b</td>
<td>421.75±279.29b</td>
<td>9.78±6.48a</td>
</tr>
<tr>
<td>King Kong</td>
<td>226±152a</td>
<td>2.00±0.91b</td>
<td>16.75±8.55b</td>
<td>618.00±471.90b</td>
<td>14.34±10.95a</td>
</tr>
<tr>
<td>Cultivar Barbaros (control)</td>
<td>393±177a</td>
<td>7.45±0.85a</td>
<td>364.56±214.93a</td>
<td>4309.00±1162.29a</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 1. Initial nematode population densities in soil (Pi), galling index (GI), number of egg masses per plant, number of eggs per gram of root, and reproduction index (RI) of *Meloidogyne arenaria* on tomato rootstocks with the Mi resistance gene in a plastic greenhouse.

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resistant to *M. incognita* A-11, but they exhibited a high resistance response to other tested populations of *M. arenaria, M. incognita*, and *M. javanica* (Aydinli & Mennan, 2019). Similarly, 'King Kong' was reported as highly resistant to both *M. arenaria* (MA-68) and *M. javanica* (MJ-05), in pot experiments under daily fluctuations of soil temperature and intermittent peaks above 28°C during the experiment (Verdejo-Lucas et al., 2013). In contrast to previous studies, 'King Kong' showed a moderate resistance to *M. arenaria* in the present study. Based on these findings, it is likely that the nematode population may have led to a reduced resistance of 'King Kong'.

The cumulative tomato fruit yield was higher for plants grafted onto rootstocks compared to nongrafted plants; however, no significant differences were detected between plants grafted onto different rootstocks (Table 2). The rootstocks provided 40.07-63.86% higher fruit yields than the susceptible control, in greenhouse infested with *M. arenaria*. This result is consistent with a few studies on tomato yields of rootstocks, in *M. incognita* and/or *M. javanica* infested greenhouses in Turkey, where grafting with 'Beaufort' provided yield increases up to 67.68% (Kaşkavalcı et al., 2009; Duran Akkurt et al., 2013). In other studies with the rootstock 'Beaufort', Lopez-Perez et al. (2006) reported two nematode-resistant rootstocks ('Hypeel45' and 'Beaufort') significantly higher fruit yields, although 'Beaufort' showed lower resistance (susceptible) to *M. incognita* than other rootstocks, in pot experiments. Moreover, total and marketable yields were greater in 'Beaufort', 'Big Power', and 'Maxifort' rootstock treatments than in nongrafted control, self-grafted control, and fumigated treatments, even if southern blight (Sclerotium rolfsii) and root-knot nematode (*M. incognita*) were both present in the same field (Rivard et al., 2010). Resistant rootstocks grown in a greenhouse infested with *M. javanica*, for three consecutive years, showed a reduced resistance level, but grafting with resistant rootstocks produced higher yield (Verdejo-Lucas et al., 2009).

These results contribute to the usefulness of resistant rootstocks as a viable component for nematode control and yield benefits in greenhouses with favorable environmental conditions for nematode development. Additionally, the rootstocks used in the present study may provide resistance to several pathogens according to information obtained from product catalogues. Thus, grafted plants onto the rootstocks could be advantageous for cost-benefit and environmental health with decreasing pesticide use, when multiple pathogens are present in the same field.

**Conclusions**

1. Rootstocks with the *Mi* resistance gene favor higher tomato fruit yields and inhibit nematode increases in soils infested with *Meloidogyne arenaria*.
2. The effectiveness of the *Mi* resistance gene is not lost in greenhouse conditions, although maximum daily ambient temperatures are above 28°C on some days during the growing period.

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**References**


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**Table 2.** Cumulative fruit yield of nongrafted (control) and grafted tomato on rootstocks with the *Mi* resistance gene, in a plastic greenhouse infested with *Meloidogyne arenaria*<sup>10</sup>.

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Cumulative yield (kg m⁻²)</th>
<th>Yield increase compared to control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arazzi</td>
<td>10.73±3.2a</td>
<td>63.86</td>
</tr>
<tr>
<td>Beaufort</td>
<td>10.11±0.82a</td>
<td>54.38</td>
</tr>
<tr>
<td>King Kong</td>
<td>9.18±4.5a</td>
<td>40.07</td>
</tr>
<tr>
<td>Cultivar Barbaros (control)</td>
<td>6.55±1.16b</td>
<td></td>
</tr>
</tbody>
</table>

<sup>10</sup>The data on yield were obtained with mean ± standard deviation of four replicated plots. Means followed by equal letters do not differ, by the Tukey’s HSD test, at 5% probability.


