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Original article (Orijinal araştırma)

Reaction of peach and nectarine rootstocks to different populations of root-knot nematode species, *Meloidogyne incognita* (Kofoid & White, 1919) and *Meloidogyne javanica* (Treub, 1885)[1]

Şeftali ve nektarin anaçlarının, Kök ur nematodu türleri; *Meloidogyne incognita* (Kofoid & White, 1919) ve *Meloidogyne javanica* (Treub, 1885)'nın farklı popülasyonlarına karşı reaksiyonu

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Abstract

The reaction of peach and nectarine rootstocks, Garnem, Cadaman, GF 677, Barrier, Nemaguard and M-29, used in Turkey was investigated to five populations of root knot nematode species, *Meloidogyne incognita* (Kofoid & White, 1919) and *Meloidogyne javanica* (Treub, 1885), under controlled conditions. The study was conducted at the Plant Protection Central Research Institute of the Laboratory of Nematology (Republic of Turkey Ministry of Agriculture and Forestry) in 2013-2016. Three *M. javanica* (TR16-2, TR12-1 and S5-1) and two *M. incognita* (TR10-3, S4-1) populations were obtained from infested peach orchards and established pure culture. All rootstocks were inoculated with 3000 second stage juveniles (J2s) from each *a* population. Each combination was replicated five times. One hundred and twenty d after inoculation, the ratio of galling on the roots and the number of nematode J2s in the soil were assessed and thus the response of rootstocks was determined. M-29, Cadaman and Garnem rootstocks were resistant to all populations, whereas GF 677 was susceptible to all populations. Nemaguard was resistant to TR16-2 and TR12-1 populations, but this rootstock was susceptible to S5-1, TR10-3 and S4-1 populations. Barrier rootstock was moderately resistant to TR16-2 and TR12 populations but susceptible to S5-1 and S4-1 populations. The findings could be used for control root-knot nematodes as well in breeding programs.

Keywords: *Meloidogyne incognita, Meloidogyne javanica*, nectarine, peach, resistance, rootstocks

Öz

Türkiye'de kullanılan Garnem, Cadaman, GF 677, Barrier, Nemaguard ve M-29 olarak adlandırılan şeftali ve nektarin anaçlarının kontrollü koşullar altında Kök ur nematodu türleri *Meloidogyne incognita* (Kofoid & White, 1919) ve *Meloidogyne javanica* (Treub, 1885)'nın beş popülasyonuna karşı reaksiyonu incelenmiştir. Çalışma, 2013-2016 yılları arasında gerçekleştirilmiştir. Çalışma, Bitki Koruma Merkezi Araştırma Enstitüsü Nematoloji Laboratuvarı (T.C. Tarım ve Orman Bakanlığı)'nda 2013-2016 yılında gerçekleştirilmiştir. Bulaşık şeftali bahçelerinden elde edilen üç *M. javanica* (TR16-2, TR12-1 ve S5-1) ve iki *M. incognita* (TR10-3, S4-1) popülasyonu ile çalışılmış olup, söz konusu popülasyonların saf kültürleri oluşturulmuştur. Bütün anaçlara her popülasyondan 3000 ikinci dönem larva (J2s) inokulasyonu yapılmıştır. Deneme, her bir anaç için beş tekerrürlü olarak kurulmuştur. Bulaştırmadan yüz yirmi gün sonra, köklerdeki ur oluşum oranı ve topraktaki ikinci dönem larva (J2s) sayısı analiz edilmiş ve böylece anaçların direnci belirlenmiştir. GF 677 tüm popülasyonlara karşı hassas iken, M-29, Cadaman ve Garnem anaçlarının tüm popülasyonlara karşı dayanıklı bulunmuştur. Nemaguard, TR16-2 ve TR12-1 popülasyonlarına dayanıklı iken, S5-1, TR10-3 ve S4-1 popülasyonlarına hassas olarak saptanmıştır. Barrier anacı TR16-2 ve TR12 popülasyonlarına karşı orta derecede dirençli iken S5-1 ve S4-1 popülasyonlarına karşı hassas olarak kaydedilmiştir. Elde edilen bulgular, kök-ur nematodlarının kontrolünde ve ıslah programlarında kullanılabilir.

Anahtar sözcükler: *Meloidogyne incognita, Meloidogyne javanica*, nektarin, şeftali, dayanıklılık, anaç

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Introduction

Turkey is among the main countries, producing peaches and nectarines in the world; China produces the most, and Turkey is the fourth largest producer (USDA, 2018). Peach-nectarine cultivation has spread to many parts of the world with mild climates. The Mediterranean basin is one of the important cultivation centers. These fruits are rich in nutrients and malic acid constitutes 80-90% of the acid found in peach. Sugars constitutes 60-65% of water-soluble dry matter. This concentration is higher in clingstone peach. One hundred g peach contains 7-12 g sugar, 2-20 mg vitamin C, nitrogen, and vitamins A and B at different ratios (Anonymous, 2008). Fruit is consumed fresh as well as jam, compote and fruit juice (Anonymous, 2004).

Today, various pest and disease problems are increasing and cause peach crop losses. Root-knot nematodes are one of the most important. Since root-knot nematodes have a very wide host range, it is difficult control. As a result of nematode feeding, large galls form throughout the root system of infected plants. Plants infested with nematodes have symptoms on the aboveground parts, including foliage yellowing and smaller leaves, because of their reduced ability to absorb and transport nutrients from the soil.

The damage varies depending on the nematode density and the sensitivity of the plant. Most plant parasitic nematodes live underground and are thus difficult to control. Therefore, plant resistance is an important management strategy. One of the most effective, environmentally friendly control measures in plants is the use of genetic host resistance. Using resistant cultivars can prevent the reproduction of the nematodes and it does not require any special application techniques or equipment, so It has a lower cost compared with other control methods (Lopez-Perez et al., 2006). The detection of the resistance of rootstock against the nematodes is very important to control the nematode and selection for establish new orchards.

There are four main root-knot nematode species, *Meloidogyne arenaria* (Neal, 1889), *Meloidogyne hapla* Chitwood, 1949, *Meloidogyne incognita* (Kofoid & White, 1919) and *Meloidogyne javanica* (Treub, 1885), that can cause damage to *Prunus* spp., but *M. incognita* and *M. javanica* are the predominant species in peach and plum (Ye et al., 2009). Root-knot nematodes cause serious problems in warm, sandy and well-drained soils (Pinochet et al., 1999). In South Carolina, *M. incognita* and *M. javanica* were found in 95 and 5% of peach orchards, respectively (Nyczepir et al., 1997). The most common symptom of root-knot nematode problems in peach is stunted growth of young trees. Thus, quantity and quality of fruit can be reduced in peach growing areas infested with RKNs. Maquilan et al. (2018) reported that RKNs caused disease complexes with fungi and bacteria in peach orchards.

Root-knot nematodes has been found on different cultivated plants in Turkey (Elekçioğlu et al., 1994; Kaşkavalcı & Öncüer, 1999; Devran & Söğüt 2009; İmren et al., 2014; Aydınlı, 2018). In Turkey, the limited studies have been conducted on peach and nectarine rootstocks. The aim of this study was to determine the reaction of the rootstocks widely used peach and nectarine production in Turkey to populations of *M. incognita* and *M. javanica*.

Material and Methods

Nematode material

Meloidogyne incognita and *M. javanica* populations were originally isolated from a peach and nectarine orchards in the Aegean Region. Soil and root samples were taken from infected peach and nectarine orchards in 2012-2013 (Yağcı et al., 2018). Three *M. javanica* (TR16-2, TR12-1 and S5-1) and two *M. incognita* (TR10-3 and S4-1) populations were obtained from these orchards (Figure 1).

Figure 1. Peach and nectarine orchards in Aegean Region.

Plant material

GF677, Garnem, M-29, Barrier, Cadaman (the clones of *Prunus cerasifera* Ehrh.) and Nemaguard (open pollinated peach seedling rootstock), which are commonly grown for peach production in Turkey, were used in this study. Tomato cv. SC 2121 was used for mass rearing of root-knot nematodes and was planted into 500 ml pots containing soil mixture. Peach materials were transplanted into 3 L pots containing sand-soil mixture previously sterilized at 120ºC (Robbins & Barker, 1974; Chen et al., 1995).

Pure culture of populations

Egg masses were collected with the help of forceps under a stereo-binocular microscope and second stage juveniles (J2s) were obtained from infested roots and the soil. They were inoculated to susceptible tomato cv. SC 2121 at the four leaf stage. After 8 weeks egg masses were collected with forceps under the stereo-binocular microscope. Pure culture of nematode populations was identified morphologically (Yağcı, 2017).

Mass rearing of nematode populations

Two *M. incognita* and three *M. javanica* populations were reared for use in this study. Experiments were conducted in a temperature-controlled glasshouse at 25-30ºC. Tomatoes were harvested and egg masses collected from roots 3 months after inoculation. The J2s were extracted from the eggs using a Baermann funnel (Hooper, 1986). About 3,000 J2s were collected under a light microscope (Leica DM 300, Wetzlar, Germany) from each population for inoculation.

Inoculation of root-knot nematode populations

Plants of uniform height of 15-20 cm and 10-20 leaves were inoculated through three 3-cm deep holes with a suspension of 3000 J2s per plant containing an equal proportion of populations (*M. incognita* and *M. javanica*) (Fernandez et al., 1995). The study was conducted at the Plant Protection Central Research Institute of the Laboratory of Nematology (Republic of Turkey Ministry of Agriculture and Forestry) in 2013-2016. Each experiment was laid out in a completely randomized design with five replicates. Plants were watered daily or as needed during the study. Experiments were conducted in a climate chamber at 25±2ºC and 65% RH with a 14:8 h L:D photoperiod. Plants were harvested 120 d after inoculation.

Data analysis

The number of galls and egg masses per root system were recorded using the 0-5 gall index scale of Hartman & Sasser (1985). Plants in scale 0 to 2 were rated as resistant, and 3 to 5 as susceptible. One-hundred-g soil samples were taken from each pot and collected to determine of the J2 density in the soil. J2s were extracted from the soil with a Baermann funnel. SPSS statistical program was used in the analysis and averages compared according to the Duncan test at $P \leq 0.01$ level.

Results and Discussion

Meloidogyne incognita and *M. javanica* were examined 120 d after inoculation and the ratio of the population densities were determined.

The reaction of rootstocks to *M. javanica* TR 16-2 was calculated according to gall index, gall number and final nematode population (Pf). M-29, Cadaman, Garnem, Nemaguard and Barrier were resistant to TR 16-2 while GF 677 was susceptible with the highest final population (Table 1).

Table 1. Number of galls, gall index and final population for *Meloidogyne javanica* population TR 16-2 on different rootstocks [(mean±SD) (min, max)], (n = 5)

Rootstock	Gall number		Gall index		Final population	
M-29	0.00±0.00 (0.00-0.00)	a*	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a
Cadaman	0.20±0.20 (0.00-1.00)	a	0.20±0.20 (0.00-1.00)	a	0.00±0.00 (0.00- 0.00)	a
Garnem	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a
Nemaguard	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a
Barrier	1.40±1.16 0.00-6.00	a	0.60±0.40 (0.00-2,00)	a	32.00±20.20 (0.00-96.00)	a
GF677	28.20±6.77 12.00-48.00	b	3.60±0.24 (3.00-4.00)	b	282.00±22.00 (216.00-340.00)	b

*Means followed by the same letter are not statistically different according to the Duncan test (P ≤ 0.01).

Rootstocks M-29, Cadaman, Garnem and Nemaguard were resistant to *M. javanica* population TR 12-1. Barrier with gall index of 2.20 in the second group was susceptible. GF 677 was susceptible with the highest number of final population and with gall index of 3.60 (Table 2).

Table 2. Number of galls, gall index and final population for *Meloidogyne javanica* population TR 12-1 on different rootstocks [(mean±SD) (min, max)], (n = 5)

Rootstock	Gall number		Gall index		Final population	
M-29	0.00±0.00 (0.00-0.00)	a*	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a
Cadaman	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00- 0.00)	a	0.00±0.00 (0.00- 0.00)	a
Garnem	0.80±0.49 (0.00-2.00)	a	0.40±0.24 (0.00-1.00)	a	0.00±0.00 (0.00-0.00)	a
Nemaguard	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a
Barrier	6.80±1,35 (3.00-11.00)	a	2.20±0.20 (2.00-3.00)	b	51.80±10.90 (30.00-90.00)	b
GF677	35.80±7.34 (9.00-52.00)	b	3.60±0.40 (2.00-4.00)	c	199.00±31.00 (81.00-256.00)	c

*Means followed by the same letter are not statistically different according to the Duncan test (P ≤ 0.01).

Rootstocks M-29, Cadaman, Garnem and Nemaguard were resistant to population TR S5-1. Barrier and GF677 rootstocks were susceptible with gall indices of 2.60 and 2.80, respectively. The highest number of J2s was found in GF677 (Table 3).

Table 3. Number of galls, gall index and final population for *Meloidogyne javanica* population S5-1 on different rootstocks [(mean±SD) (min, max)], (n = 5)

Rootstock	Gall number		Gall index		Final population	
M-29	0.00±0.00 (0.00-0.00)	a*	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a
Cadaman	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a
Garnem	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a
Nemaguard	3.60±1.83 (0.00-9.00)	a	1.20±0.37 (0.00-2.00)	ab	35.60±20.00 (0.00-108.00)	a
Barrier	26.80±12.00 (2.00-72.00)	ab	2.60±0.49 (1.00-4.00)	c	182.00±47.50 (30.00-314.00)	c
GF677	53.00±22.30 (0.00-107.00)	b	2.80±1.07 (0.00-5.00)	b	223.00±93.70 (0.00-441.00)	c

*Means followed by the same letter are not statistically different according to the Duncan test (P ≤ 0.01).

Rootstocks M-29, Cadaman and Garnem were resistant to *M. incognita* population S4-1. Nemaguard, Barrier and GF677 were susceptible with gall indices of 4.00, 3.80 and 4.80. The highest final population was found in GF677, no J2s were found in M-29 and Garnem (Table 4).

Table 4. Number of galls, gall index and final population for *Meloidogyne incognita* population S4-1 on different rootstocks [(mean±SD) (min, max)], (n = 5)

Rootstock	Gall number		Gall index		Final population	
M-29	0.00±0.00 (0.00-0.00)	a*	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a
Cadaman	1.60±1.60 (0.00-8.00)	a	0.40±0.40 (0.00-2.00)	a	11.80±11.80 (0.00-59.0)	a
Garnem	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a
Nemaguard	71.80±24.80 (4.00-131)	b	4.00±0.54 (2.00-5.00)	bc	182.00±30.20 (95.00-282.00)	b
Barrier	52.80±9.10 (18.00-68.00)	b	3.80±0.20 (3.00-4.00)	b	287.00±28.30 (204.00-370.00)	c
GF677	115.00±17.60 (70.00-178.00)	c	4.80±0.20 (4.00-5.00)	c	459.00±52.30 (269.00-583.00)	d

*Means followed by the same letter are not statistically different according to the Duncan test (P ≤ 0.01).

Rootstocks Cadaman and Garnem were resistant to *M. incognita* population TR10-3. Nemaguard and GF677 were susceptible with gall indices of 3.00 and 5.00, respectively. Final population score of Cadaman and Garnem were 0, whereas GF677 was 554 (Table 5).

Table 5. Number of galls, gall index and final population for *Meloidogyne incognita* population TR10-3 on different rootstocks [(mean±SD) (min, max)], (n = 5)

Rootstock	Gall number		Gall index		Final population	
Cadaman	0.00±0.00 (0.00-0.00)	a*	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a
Nemaguard	21.70±2.96 (16.00-26.00)	a	3.00± 0.00 (3.00-3.00)	a	110.00±18.70 (89.00-147.00)	a
Garnem	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a	0.00±0.00 (0.00-0.00)	a
GF677	151.00±21.90 (113.00-189.00)	b	5.00±0.00 (5.00-5.00)	b	554.00±66.20 (433.00-661.00)	b

*Means followed by the same letter are not statistically different according to the Duncan test (P ≤ 0.01).

Rootstocks M-29, Cadaman, Garnem, Nemaguard and Barrier were resistant to TR 16-2 while GF 677 was susceptible with the highest final population. GF 677 and Barrier were susceptible to *M. javanica* population TR 12-1 and M-29, Cadaman, Garnem and Nemaguard resistant. Whereas, M-29, Cadaman, Garnem and Nemaguard were resistant to TR S5-1 and Barrier and GF677 were were susceptible. M-29, Cadaman and Garnem were resistant to *M. incognita* population S4-1 and Nemaguard, Barrier and GF677 were susceptible. Cadaman and Garnem were resistant to *M. incognita* population TR10-3 and Nemaguard and GF677 were susceptible (Figure 2). Yoshikawa et al. (1989) reported that Nemaguard, Nemared and Lovell rootstocks were widely used in California, and Nemaguard and Nemared rootstocks were resistant to root-knot nematodes.

Rootstocks M-29, Cadaman and Garnem were resistant to all nematode populations. However, Pinochet et al. (1999) found that Garnem and Cadaman were resistant to *M. javanica*. Likewise, Özbek et al. (2014) reported that Garnem, Cadaman and Myrobalan 29-C when inoculated J2s of *M. incognita* race 2 and *M. javanica* all rootstocks were resistant. Esmenjaud et al. (1997) reported that M-29 was resistant to populations of *M. arenaria, M. incognita* and *M. javanica*. In another study, Özarslandan & Tanrıver (2018) showed that Myrobalan 29-C, Garnem, Patrones Arda, Cadaman, Patrones Toro, Mariana GF 8-1 rootstocks were resistant to *M. incognita* while Myrobalan B and GF677 were susceptible.

Figure 2. Galls on the peach and nectarine roots caused by *M. incognita*.

Nemaguard and Barrier are reported to be resistant to root-knot nematodes (Sherman & Lyrene, 1983; Huettel & Hammerschlag, 1993; Pinochet et al., 1996, 1999; Layne & Bassi, 2008). However, in the present study these rootstocks were susceptible to some of nematode populations. Similarly, Esmenjaud et al. (1997) reported that Nemaguard was resistant to populations of *M. arenaria, M. incogn*ita and *M. javanica* but it was susceptible to Florida isolates (*M. incognita* race 3). Meza et al. (2016) showed that Nemaguard had variable resistance to each of the most aggressive isolates.

GF 677 was susceptible to all root-knot nematode populations. In previous studies, Pinochet al. (1996) showed that GF677 was susceptible to *M. incognita* (5 populations), *M. javanica* (5 populations), *M. arenaria* (5 populations), *M. hapla* (1 population), *Meloidogyne hispanica* Hirschmann, 1986 (1 population) and Barrier was moderately resistant. Cadaman and Nemaguard rootstocks were also resistant to all populations. In another study, Fernandez (1995) reported that GF677 was susceptible while Barrier was moderately resistant to *M. incognita.* Nyczepir & Wood (2012) reported that Nemaguard was highly resistant to *Meloidogyne partityla* Kleynhans, (1986) and no egg masses were present on the roots. Additionally, Marull et al. (1991) reported that GF-677 was susceptible to *M. arenaria.*

Several studies have been conducted on *Prunus*. Esmenjaud et al. (1995) found that in their study 15-month-old hardwood cuttings of Myrobalan (*P. cerasifera*) were resistant to *M. arenaria* populations. In another study, Ye et al. (2009) showed that root-knot nematodes caused serious damage to the *Prunus* rootstocks in China and some cultivars such as Tsukuba-4 and Tsukuba-5 were immune to *M. incognita*.

In conclusion, Barrier, Nemaguard and GF 677 rootstocks should not be selected to establish new orchards in the Aegean Region of Turkey. Garnem, M-29 and Cadaman rootstocks are resistant to all root-knot nematode populations in our study. Thus, they should be used in the peach and nectarine cultivation. These resistant rootstocks can be used to control *M. incognita* and *M. javanica* in IPM programs. The results could provide important knowledge for plant breeders, and peach and nectarine growers.

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