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Original article (Orijinal araştırma)

Host suitability of pepper cultivars to (a)virulent root-knot nematodes isolates

Biber çeşitlerinin (a)virulent kök-ur nematod izolatlarına karşı konukçuluk durumu

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Abstract

Root-knot nematodes (RKNs) are polyphagous and cause yield losses to crops worldwide. Using the resistant plant is the most effective and environmental method to manage RKNs. *Mi-1.2* gene in tomatoes is commonly used to control *Meloidogyne*. However, the *Mi-1.2*-virulent isolates can overcome this gene. In fields infested with *Mi-1.2*-virulent populations, plant species with different resistance genes are recommended instead of tomatoes. Therefore, investigating the host suitability of pepper cultivars to *Mi-1.2*-virulent RKNs is needed for management practices. This study was conducted in Akdeniz University Faculty of Agriculture Department of Plant Protection Nematology Laboratory in 2019. In this study, the response of resistant and susceptible pepper cultivars was investigated to *Meloidogyne incognita (*Kofoid & White,1919), Chitwood, 1949*, Meloidogyne javanica* (Treub, 1885) Chitwood, 1949*, Meloidogyne arenaria (*Neal, 1889) Chitwood, 1949, *Meloidogyne luci* Carneiro et al., 2014 (Tylenchida: Meloidogynidae)*, Mi-1.2*-virulent *M. incognita* and *Mi-1.2*-virulent *M. javanica. Meloidogyne arenaria*, *M. incognita* and *Mi-1.2*-virulent *M. incognita* isolates multiplied very well on susceptible peppers but did not reproduce on resistant peppers. *Mi-1.2*-(a)virulent *M. javanica* isolates did not multiply on any pepper cultivars. *Meloidogyne luci* reproduced on all peppers tested. A pepper carrying *N* resistance gene was first tested with *M. luci* and did not confer resistance to *M. luci*. These results could be used to control RKNs in vegetable-growing areas.

Keywords: *Capsicum annum*, *Meloidogyne*, *Mi-1.2*-virulent, *N* gene, resistance

Öz

Kök-ur nematodları polifag olup tüm dünyada tarım ürünlerinde verim kayıplarına neden olurlar. Dayanıklı bitki kullanımı kök-ur nematodları ile mücadelede en etkili ve çevreci yöntemdir. Ancak, *Mi-1.2*-virülent kök-ur nematod popülasyonları domatesteki dayanıklılığı kırabilmektedir. *Mi-1.2*-virülent popülasyonlarla bulaşık arazilerde domates yerine farklı dayanıklılık genleri taşıyan bitki türleri önerilmektedir. Bu nedenle biber çeşitlerinin *Mi-1.2*-virülent kök-ur nematodlarına konukçuluk durumunun araştırılması kök-ur nematodları ile mücadele için gereklidir. Bu çalışma 2019 yılında Akdeniz Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü Nematoloji laboratuvarında yürütülmüştür. Çalışmada dayanıklı ve hassas biber çeşitlerinin *Meloidogyne incognita* Kofoid & White,1919), Chitwood, 1949, *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, *Meloidogyne luci* Carneiro et al., 2014 (Tylenchida: Meloidogynidae)*, Mi-1.2*-virülent *M. incognita* ve *Mi-1.2*-virülent *M. javanica*'ya karşı tepkisi incelenmiştir. *Meloidogyne arenaria*, *M. incognita* ve *Mi-1.2*-virülent *M. incognita* izolatları hassas biber çeşitlerinde ürerken dayanıklı çeşitlerde üremenmiştir. *Mi-1.2*-(a)virulent *M. javanica* izolatları hiçbir biber çeşidinde çoğalamamıştır. *Meloidogyne luci*, testlenen tüm biber çeşitlerinde çoğalmıştır. *N* dayanıklılık geni taşıyan biberler ilk kez *M. luci* ile testlenmiş ve *M. luci*'ye dayanıklılık sağlamamıştır. Bu sonuçlar sebze yetiştiriciliği yapılan alanlarda kök-ur nematodlarla mücadelede faydalı olabilecektir.

Anahtar sözcükler: *Capsicum annum*, *Meloidogyne*, *Mi-1.2*-virülent, *N* geni, dayanıklılık

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Introduction

Root-knot nematodes (RKNs) are organisms that are difficult to manage due to their short generation times and high reproduction rates. The use of genetic resistance to RKNs provides efficient, economically competitive, and eco-friendly management (Devran et al., 2010). Some resistance genes against RKNs have been described from Solanaceae (Smith, 1944; Hendy et al., 1985; Fery et al., 1998). In tomato, *Mi-1.2* gene is the most important resistant gene for controlling RKNs and is widely used in commercial tomato cultivars (Devran & Söğüt, 2014; Seid et al., 2015). *Mi-1.2* gene provides effective protection against the three most destructive RKNs, *Meloidogyne incognita* Kofoid & White,1919), Chitwood, 1949*, Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 and *Meloidogyne javanica (*Treub, 1885) Chitwood, 1949 (Tylenchida: Meloidogynidae) *(*Williamson & Hussey, 1996) as well as other RKN species (Aydınlı & Mennan, 2019; Gabriel et al., 2020; Santos et al., 2020). However, the resistance mediated by *Mi-1.2* is lost at high soil temperatures and can be overcome by *Mi-1.2*-virulent RKN isolates (Dropkin, 1969; Kaloshian et al., 1996; Tzortzakakis et al., 2005; Devran & Söğüt, 2010; Özalp & Devran, 2018). Since *Mi-1.2*-virulent isolates have become common in vegetable fields, alternating the different resistance genes may be an effective management strategy against virulent populations. Thus, there is a need to understand the reactions of different resistance genes to *Mi-1.2*-virulent RKN populations

Pepper (*Capsicum annum* L.) (Solanales: Solanaceae) is an economically important member of the Solanaceae family. In pepper, several genes controlling resistance to RKNs have been determined. From these genes *N*, *Me1, Me3* and *Me7* confer resistance to three most destructive RKNs (Djian-Caporalino et al., 1999; Castagnone-Sereno et al*.,* 2001). Resistant pepper cultivars are used for controlling RKNs (Barbary et al., 2015). In addition, resistant pepper cultivars are considered as rotation crops in the cultivation of susceptible vegetables for *Meloidogyne* (Thies et al., 1998). Thus, it is very important to know the responses of peppers with and without resistance genes against RKNs obtained from different locations**.**

Studies show that the reproductive potential of *Mi-1.2*-virulent RKN isolates on susceptible pepper cultivars is lower than that of avirulent isolates. Djian-Caporalino et al. (2011) observed that virulent RKN populations against the *Mi-1*, *Me1* and *Me3* resistance genes had lower reproduction on susceptible tomato and pepper plants. In this case, knowing the response of *Mi-1.2*-virulent isolates in different susceptible pepper cultivars can be important for crop rotation programs.

The aims of this study were i) to compare the response of pepper cultivars to three most destructive RKNs and *Meloidogyne luci* Carneiro et al., 2014 (Tylenchida: Meloidogynidae) isolates, ii) determine the effectiveness of *N* gene to (a)virulent RKN isolates, and iii) designate whether the *N* gene confers resistance to *M. luci*.

Materials and Methods

Plant materials

Pepper cultivars Safran F1, Mostar F1 and Mert F1 were used as susceptible to RKNs. The resistant pepper line Coralina Wonder is homozygous for the *N* gene. Responses to RKNs of pepper cultivars B5 line, B6 line and B4 F1 (B5xB6) and local accession KB401 are unknown. The seeds of KB401 were obtained from a local farmer, while other pepper varieties were provided from Yüksel Tohum (Antalya, Türkiye).

Nematode isolates

RKNs isolates used in the experiments are listed in Table 1. Pure cultures were reproduced from single egg mass. *Mi-1.2* natural virulent and avirulent isolates were reproduced on resistant and susceptible tomato cultivars, respectively.

Table 1. *Meloidogyne* isolates used in the experiments

Code	Nematode species	Property	Reference
S6	*Meloidogyne incognita*	Avirulent	Devran & Sögüt, 2009
K21	*Meloidogyne javanica*	Avirulent	Devran & Sögüt, 2009
K18	*Meloidogyne arenaria*	Avirulent	Devran & Sögüt, 2009
Tk4	*Meloidogyne luci*	Avirulent	Aydınlı & Mennan, 2019
V19	*Meloidogyne incognita*	*Mi-1.2*-virulent	Mıstanoğlu et al., 2020
V24	*Meloidogyne incognita*	*Mi-1.2*-virulent	Mıstanoğlu et al., 2020
V23	*Meloidogyne javanica*	*Mi-1.2*-virulent	Mıstanoğlu et al., 2020
V28	*Meloidogyne javanica*	*Mi-1.2*-virulent	Mıstanoğlu et al., 2020

The testing of peppers

For all the nematode isolates, egg masses were collected from infected roots and hatched at 25°C. Second-stage juveniles (J2s) were collected from water every 48 hours and counted under the binocular stereo microscope. Each cultivar seedling at the four-leaf stage was transplanted into 250 cc plastic cups including autoclaved sandy soil mixture. 7 days after transplanting, each plant was separately inoculated with 1000 J2s of RKN isolates. The plants were incubated in a controlled growth chamber at 25°C, 65% relative humidity and 16:8 hours day/night cycle. The plants were gently uprooted 8 weeks after J2s inoculation and roots were cleaned from soil individually using tap water. There were five replicates for each combination (isolate x cultivar) according to a completely randomized design. Each root system was stained with Phloxine B solution for counting egg masses and investigated under a microscope (Öçal et al., 2018).

Data analyses

The data were analyzed using ANOVA with the statistical package SAS v. 9.0. Significant differences within nematode isolates and pepper cultivars were analyzed separately using Duncan's multiple range test ($p \leq 0.05$).

Results and Discussion

In the first experiment, pepper cultivars Safran F_1, Carolina Wonder, Monstar F_1, B4 F1 and KB401 were inoculated with avirulent RKN isolates, *M. incognita* (S6), *M. javanica* (K21), *M. arenaria* (K18) and *M. luci* (Tk4). Except for *M. javanica* K21 isolate, all RKN isolates reproduced on Safran F_1 and Mostar F_1 were known to be susceptible. *Meloidogyne javanica* K21 produced few or no egg masses on pepper cultivars. *M. luci* isolate produced the most egg masses Carolina Wonder genotype bearing *N* gene and B4 F_1. Except *M. javanica,* all RKN isolates produced egg masses on local cultivar KB401. *Meloidogyne luci* multiplied less when compared with the other isolates on KB401, indicating that it may not be a good host for *M. luci*. As expected, three main RKN isolates did not reproduce on resistant Carolina Wonder (Table 2).

Table 2. The average number of egg masses of *Mi-1.2*-avirulent isolates on the whole root system in pepper cultivars

Pepper cultivars	Nematode species and their codes							
	M. incognita (S6)		*M. javanica* (K21)		*M. arenaria* (K18)		*M. luci* (Tk4)	
Safran F1	111.2±8.4	Ab[x, y]	0.2±0.2	Ca	32.6±1.9	Ba	38.0±7.8	Bb
Carolina Wonder	4.4±0.9	Bc	0.0±0.0	Ba	0.6±0.6	Bc	68.4±5.9	Aa
Mostar F1	126.2±13.9	Bab	0.2±0.2	Da	36.0±5.1	Ca	27.2±3.7	CDb
B4 F1	7.4±3.5	Bc	0.0±0.0	Ca	0.0±0.0	Cc	31.8±2.6	Ab
KB401	147.6±12.0	Ba	0.0±0.0	Ca	13.8±4.6	Cb	8.8±2.1	Cc

[x] Means (± standard error) in a column with the same lower-case letter are not significantly different in accordance with Duncan's multiple range test ($p \leq 0.05$).

[y] Means (± standard error) in a row with the same upper-case letter are not significantly different in accordance with Duncan's multiple range test ($p \leq 0.05$).

In the second experiment, pepper cultivars Safran F_1, Monstar F_1, Mert F_1, Carolina Wonder, B4 F_1, B5 and B6 were inoculated with *Mi-1.2*-virulent *M. incognita* isolates V19 and V24 and *Mi-1.2*-virulent *M. javanica* isolates V28 and V23. *Mi-1.2*-virulent *M. incognita* isolates caused high numbers of egg masses and galls on susceptible pepper cultivars Safran F_1, Mostar F_1 and Mert F_1, but reproduced no egg masses and galls on resistant cultivars Carolina Wonder and B4 F_1. Similarly, *Mi-1.2*-virulent *M. incognita* isolates caused egg masses on B5 but did not on B6. *Mi-1.2*-virulent *M. javanica* isolates did not multiply on any of the pepper cultivars (Table 3).

Table 3. The average number of egg masses of *Mi-1*-virulent RKN isolates in pepper cultivars

Pepper cultivars	Nematode species and their codes							
	M. incognita (V19)		*M. incognita* (V24)		*M. javanica* (V28)		*M. javanica* (V23)	
Safran F1	64.8±9.8	Bb [x, y]	120.4±18.5	Aab	0.0±0.0	Ca	0.0±0.0	Ca
Mostar F1	94.8±23.9	Aab	102.6±6.0	Ab	0.0±0.0	Ba	0.2±0.2	Ba
Mert F1	107.6±11.2	Aa	135.6±16.9	Aa	0.0±0.0	Ba	0.4±0.4	Ba
Carolina Wonder	0.0±0.0	Ac	0.0±0.0	Ad	0.0±0.0	Aa	0.0±0.0	Aa
B4 F1	0.2±0.2	Ac	0.0±0.0	Ad	0.0±0.0	Aa	0.0±0.0	Aa
B5	65.6±7.9	Ab	70.8±11.1	Ac	0.0±0.0	Ba	0.0±0.0	Ba
B6	0.0±0.0	Ac	0.0±0.0	Ad	0.0±0.0	Aa	0.2±0.2	Aa

[x] Means (± standard error) in a column with the same lower-case letter are not significantly different in accordance with Duncan's multiple range test ($p \leq 0.05$).

[y] Means (± standard error) in a row with the same upper-case letter are not significantly different in accordance with Duncan's multiple range test ($p \leq 0.05$).

Overall, in this study, avirulent *M. arenaria* and (a)virulent *M. incognita* isolates multiplied very well on susceptible peppers (Safran F1, Mostar F1 and Mert F1) but did not on Carolina Wonder. However, (a)virulent *M. javanica* isolates did not multiply all pepper cultivars tested. *M. luci* isolate was multiplied all pepper cultivars except for KB401. Pot tests showed that B4 F_1 were resistant to *Mi-1.2*-virulent and avirulent isolates of *M. incognita,* and *M. javanica* as well as avirulent isolate of *M. arenaria.* B4 F1 was obtained from crossing B6 with B5. Thus, the resistance in B4 F1 come from B6 line.

Castagnone-Sereno et al. (1992) reported that *Mi-1.2*-virulent RKNs lose their reproductive ability on susceptible pepper cultivars, surprisingly. A later study by Castagnone-Sereno et al. (2001) found that the *Mi-1.2*-virulent RKN populations were less able to reproduce on susceptible pepper cultivars, with few exceptions. *Mi-1.2*-virulent *M. incognita* populations collected from Greece could reproduce on resistant tomatoes but did not on susceptible pepper (Tzortzakakis & Blok, 2007). In another study, *Mi-1.2*-virulent *M. incognita* population generally failed to reproduce on susceptible pepper. In contrast to this finding, another *Mi-1.2*-virulent *M. incognita* population was able to reproduce on susceptible pepper (Tzortzakakis et al., 2016). In the present study, *Mi-1.2*-virulent *M. incognita* isolates developed on susceptible pepper cultivars but did not multiply on resistant Caroline Wonder, also B4 F_1 and B6 cultivars.

Caroline Wonder bearing *N* gene was resistant to *M. incognita*, *M. javanica*, *M. arenaria, Mi-1.2*-virulent *M. incognita* and *Mi-1.2*-virulent *M. javanica* isolates but was susceptible to *M. luci.* Our results related to *M. incognita*, *M. javanica*, *M. arenaria* are consistent with previous studies (Thies et al., 1998; Thies & Fery, 2000). To our best knowledge, no report exists on the response of *N* gene in pepper against *M. luci*. The study is the first to investigate the interaction between *M. luci* and *N* resistance gene. Results showed that *N* gene is susceptible to *M. luci* isolate tested.

As known, *M. luci* is one of the important RKNs due to its spread in Europe (Maleita et al., 2018). *Meloidogyne ethiopica* populations identified in Europe were later reidentified to be *M. luci (*Gerič Stare et al., 2017). *Meloidogyne luci* has been on the European Plant Protection Organization Warning list since 2017 (EPPO, 2017). However, there are limited studies on the host responses of plants to *M. luci* (Carneiro et al., 2014; Aydınlı & Mennan, 2016, 2019; Öçal et al., 2018; Şen & Aydınlı, 2021; Maleita et al., 2022). Carneiro et al. (2014) reported that *M. luci* can reproduce on susceptible pepper California Wonder. Şen & Aydınlı

(2021) found that one of the five pepper cultivars they tested was a poor host for *M. luci*. Maleita et al. (2022) investigated the response of ten pepper cultivars to *M. luci* and detected all of them as susceptible. In this study, *M. luci* multiplied on susceptible peppers Safran F_1 and Mostar F_1. However, *M. luci* developed less than *M. incognita* on susceptible peppers. Also, *M. luci* developed at least on KB401 cultivar. *Meloidogyne luci* was commonly present in the northeast of Türkiye (Aydınlı & Mennan, 2016; Aydınlı, 2018). Besides, *M. luci* was found in Osmaniye, southeast of Türkiye (Gürkan et al., 2019). Our results showed that pepper cultivars that are not good hosts for *M. luci* may be used in areas infected with *M. luci.* Since tomato cultivars bearing the *Mi-1* gene and *Solanum torvum* Sw. (Solanales: Solanaceae) rootstock have been found resistant against *M. luci* in previous studies, these can be recommended for infected areas (Sargın & Devran, 2021).

It is recommended to alternate resistance genes to maintain sustainability of resistant varieties and to prevent virulent populations (Barbary et al., 2015). In this respect, results provide useful information on response of pepper cultivars to important RKNs, *M. incognita*, *M. javanica*, *M. arenaria* and *M. luci.*

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