

Original article (Orijinal araştırma)

The response of the pepper with and without *Me1* gene to *Mi-1.2*-virulent *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) isolates¹

Me1 geni taşıyan ve taşımayan biberlerin *Mi-1.2*-virulent *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) izolatlarına tepkisi

Ahmet Kaan AKSAN² 

Tevfik ÖZALP³ 

Zübeyir DEVRAN^{4*} 

Abstract

Root-knot nematodes are important organisms that infect vegetables. Due to the intense use of *Mi-1.2*, virulent populations that break resistance have become widespread and have become an important factor limiting the use of this gene. *Me1* resistance gene on pepper provides resistance against *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Tylenchida: Meloidogynidae) species. However, there is limited information on the effectiveness of the *Me1* gene against *Mi-1.2* virulent populations. Therefore, it is important to know the reaction of pepper cultivars carrying the *Me1* resistance gene against *Mi-1.2* virulent populations. In this study, the response of resistant pepper cultivar MT-01 F1 (bearing *Me1*) and susceptible pepper cultivar Safran F1 against both *Mi-1.2* natural virulent *M. incognita* isolates and *Mi-1.2* selected virulent *M. incognita* isolate was investigated under controlled conditions. This study was conducted in Akdeniz University Faculty of Agriculture Department of Plant Protection Nematology Laboratory in 2021. All isolates caused many egg masses and galls on the resistant tomato cultivar Seval F1 as expected, and the susceptible pepper cultivar Safran F1. Five isolates were found to cause egg masses and gall formation, while the V3 isolate did not multiply on the resistant pepper cultivar MT-01 F1. The results showed that pepper cultivars carrying the *Me1* gene exhibited different responses against *Mi-1.2* virulent isolates.

Keywords: *Capsicum annum*, resistance, RKN, root-knot nematode

Öz

Kök-ur nematodları sebzelerde zarar yapan önemli organizmalardır. *Mi-1.2* geninin yoğun kullanımı nedeniyle, bu genin sağladığı dayanıklılığı kıran virulent popülasyonlar yaygınlaşmış ve bu genin kullanımını sınırlayan önemli bir faktör haline gelmiştir. Biberdeki *Me1* dayanıklılık geni, *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949; *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 ve *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Tylenchida: Meloidogynidae) türlerine koruma sağlamaktadır. Ancak, *Me1* geninin *Mi-1.2* virulent popülasyonlarına karşı performansı hakkında detaylı bilgi bulunmamaktadır. Bu yüzden, *Me1* genini taşıyan biberlerin *Mi-1.2* virulent popülasyonlarına tepkisinin bilinmesi önemlidir. Bu çalışmada, *Mi-1.2* doğal virulent *M. incognita* izolatlarına ve *Mi-1.2* seçilmiş virulent *M. incognita* izolatına karşı *Me1* geni taşıyan dayanıklı biber çeşidi MT-01 F1 ve duyarlı biber çeşidi Safran F1'in tepkisi kontrollü koşullar altında araştırılmıştır. Bu çalışma 2021 yılında Akdeniz Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü Nematoloji laboratuvarında yürütülmüştür. Tüm izolatlar, beklediği gibi dayanıklı domates çeşidi Seval F1 ve duyarlı biber çeşidi Safran F1 üzerinde çok sayıda yumurta kümesi ve ur oluşturmuştur. Beş izolatın dayanıklı biber çeşidi MT-01 F1 üzerinde yumurta kümesi ve ur oluşumuna neden olduğu, ancak V3 izolatının çoğalmadığı tespit edilmiştir. *Me1* geni taşıyan biber çeşidinin *Mi-1.2* virulent izolatlarına karşı farklı tepkiler gösterdiği belirlenmiştir.

Anahtar sözcükler: *Capsicum annum*, dayanıklılık, RKN, kök-ur nematodu

¹ Data in this article was derived from first author's Master Thesis.

² Akdeniz University, Institute of Natural and Applied Sciences, Department of Plant Protection, 07100, Konyaaltı, Antalya, Türkiye

³ Directorate of Plant Protection Research Institute, 35040, Bornova, İzmir, Türkiye

⁴ Akdeniz University, Faculty of Agriculture, Department of Plant Protection, 07100, Konyaaltı, Antalya, Türkiye

* Corresponding author (Sorumlu yazar) e-mail: zdevran@akdeniz.edu.tr

Received (Alınış): 30.04.2024

Accepted (Kabul edilmiş): 27.08.2024

Published Online (Çevrimiçi Yayın Tarihi): 28.08.2024

Introduction

Pepper, *Capsicum* spp. (L.) (Solanales: Solanaceae) is an economically important vegetable species. However, Root-knot nematodes (RKNs) cause economic losses in pepper production areas (Talavera-Rubia et al. 2022). One of the most effective control methods against RKNs is using resistant cultivars which prevent nematode feeding and reproduction on plant roots (Lopez-Perez et al., 2006). *Mi-1.2* in tomatoes, which is one of the most commonly used genes to plant, provides resistance against the major RKN species, *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Tylenchida: Meloidogynidae) (Williamson & Hussey, 1996). However, gene inactivation at high soil temperatures (Dropkin, 1969; Özalp & Devran, 2018) and the ability of *Mi-1.2* virulent populations to break resistance are factors limiting its use (Roberts, 1990). *Mi-1.2* virulent RKN populations are becoming increasingly common. Virulent populations breaking *Mi-1* resistance have been reported in many countries around the world, including the USA (Riggs & Wingstead, 1959), India (Sikora et al., 1973), Senegal (Berthou et al., 1989); Japan (Narabu & Momota, 1992), Tunisia (Eddaoudi et al., 1997), Spain (Ornat et al., 2001), France (Jacquet et al., 2005), Greece (Tzortzakakis et al., 2005), Türkiye (Devran & Söğüt, 2010), Israel (Iberkleid et al., 2014), Brazil (Silva et al., 2019). Therefore, using different resistance genes in fields where *Mi-1.2* virulent populations are detected is recommended. In addition, knowing the reproductive potential of *Mi-1.2* virulent RKNs in vegetables other than tomatoes would also be valuable information for crop rotation.

Many resistance genes against RKNs have been detected in pepper, making it a vegetable species with high economic value that can be used in crop rotation. The *Me* and *N* genes are used in breeding programs against RKNs in pepper. *Me1*, *Me3*, *Me7* and *N* genes are effective against *M. arenaria*, *M. incognita*, and *M. javanica*, which are common species in pepper production areas (Djian-Caporalino et al., 1999, 2001, 2007; Changkwian et al., 2019). In addition, it has been shown that *N*, *Me1* and *Me3* genes provide resistance against *Meloidogyne haplanaria* Eisenback et al., 2003 (Tylenchida: Meloidogynidae) as well as the three major species (Hajihassani et al., 2019). The *N* gene carrying pepper cultivar Carolina Wonder was recently tested against *Meloidogyne luci* Carneiro et al., 2014 (Tylenchida: Meloidogynidae), and it was found that the *N* gene does not confer resistance to *M. luci* (Özalp et al., 2024). Because of the potential of resistance genes in pepper, it is essential to know the responses of resistance genes against *Mi-1.2* virulent RKN populations. Therefore, studies have been conducted on the responses of some genes conferring resistance to RKNs in pepper against *Mi-1.2* virulent populations (Castagnone-Sereno et al., 1992, 2001; Tzortzakakis & Blok, 2007; Djian-Caporalino et al., 2011; Özalp et al., 2024).

However, to better understand the responses of pepper cultivars carrying the resistance gene against RKNs, further studies and determination of the host status of these peppers against virulent RKN isolates obtained from different locations are required. Therefore, this study aims to investigate the response of a pepper cultivar carrying the *Me1* gene against the selected and natural *Mi-1.2* virulent *M. incognita* isolates from Türkiye.

Materials and Methods

Plant materials

The susceptible pepper cv. Safran F1 and resistant pepper cv. MT-01 F1 carrying the *Me1* gene were used in this study. In addition, the resistant tomato cv. Seval F1, bearing the *Mi-1.2* gene, was used as control in the experiments. Safran F1, MT-01 F1 and Seval F1 seeds were provided from vegetable seed companies, which are Yuksel Seeds and Multi Seeds, Antalya, Türkiye.

Nematode populations

In the study, *Mi-1.2* virulent *M. incognita* isolates (V3, V6, V13, V15, V18 and V30) were used, five of which were naturally *Mi-1.2* virulent, and one (V30) was selected for virulence in the laboratory. The *Mi-1.2* natural virulent isolates were collected from protected greenhouses with sandy soil types growing resistant tomato cultivars in Kepez district of Antalya province of Türkiye. The *Mi-1.2* natural virulent isolates V6 and V18 were used in previous studies (Mıstanoğlu et al., 2020; Sargın & Devran, 2021). Pure cultures were multiplied from a single egg mass. In addition, the *Mi-1.2* selected virulent V30 isolate was obtained from the continuous selection of an avirulent *M. incognita* isolate on a resistant tomato cv. under controlled conditions (Mıstanoğlu, 2020). For this purpose, 1000 J2s avirulent *M. incognita* from the S6 isolate was inoculated onto cv. Seval F1. After the first inoculation, a very few egg masses (2 to 5) were obtained from plant roots. All juveniles obtained from these egg masses were re-inoculated until the population became *Mi-1.2* virulent, which took approximately five generations. By the time the experiment in this paper was set up, the V30 isolate had been multiplied at least 10 times on the resistant tomato.

Culturing of the *Mi-1.2* virulent isolates

The *Mi-1.2* virulent isolates were maintained on resistant tomato cv. Seval F1. For this purpose, the Seval F1 seeds were sown in vials and maintained in a controlled condition at $25\pm 1^{\circ}\text{C}$. Then, for each isolate, tomato seedlings were planted in 250 ml plastic pots. For the multiplication of each isolate, twenty plants were separately inoculated with egg masses of each isolate after 7 days from planting. Plants were cultivated in a controlled growth chamber as in previous studies (Öçal et al., 2018; Özalp & Devran, 2018). Following nematode inoculation, plants were harvested from their pots 60 days later and their roots were carefully washed under running tap water to remove any adhering soil. Subsequently, individual egg masses were meticulously extracted from visibly galled roots using a sterile needle. To promote hatching, these egg masses were then incubated within a sieve apparatus maintained at 25°C room temperature for 24 hours. Subsequently, the hatched J2s were treated using established methodologies outlined in previous studies (Öçal et al., 2018; Özalp & Devran, 2018)

Confirmation of *Mi-1.2* virulent isolates

In order to compare the reproduction of isolates on Seval F1, nematode testing was conducted under controlled conditions. For this purpose, Seval F1 seedlings with the four-leaf stage were transplanted into 250 ml pots using autoclaved sandy soil. After one week, each plant was inoculated with 1000 J2s. Each isolate was inoculated to five plants. Therefore, there were five replicates for each isolate. The experiment was performed as two repeats according to a completely randomized design. The plants were grown in a controlled climate cabin with a temperature of $25\pm 1^{\circ}\text{C}$, a humidity of $60\pm 5\%$, and 16:8 hours light-dark cycle. Plants were uprooted eight weeks after inoculation.

The testing of pepper plants

The resistant pepper MT-01 F1 which carries the *Me1* gene and susceptible pepper Safran F1 were used in this experiment. For this purpose, both pepper cultivar seeds were sown in vials and maintained in an environment condition at $25\pm 5^{\circ}\text{C}$. Each cultivar seedling at the four-leaf stage were transplanted into 250 cc plastic pots containing sterile soil. One week after transplanting the plants, 1000 J2s per plant were inoculated for each isolate. There were five replicates for each combination (isolate x cultivar) according to a completely randomized design. The experiment was repeated two times (10 replications in total). The plants were grown in a growth chamber with a temperature of $24\pm 1^{\circ}\text{C}$ and humidity of $60\pm 5\%$, and 16:8 hours light-dark cycle.

Data evaluation

Approximately eight weeks after inoculation, the experiment was finalized, and plants were uprooted. The roots of the uprooted pepper plants were washed, and their root weights were saved. Then, the roots were stained with Phloxine B (Merck) (0.15 g/L) for easier counting of egg masses (Öçal et al., 2018). Egg masses and galls on the roots were counted, and the resistant status of the plants was scored according to the 0-5 index (Hartman & Sasser 1985). Egg mass index and gall index were evaluated according to all root system. The reproduction factor [RF= number of J2 in soil at the final + number of eggs in egg masses (Pf) / initial population density of nematodes (Pi)] value of the *M. Incognita* V3 isolate was calculated to determine the multiplication status in resistant and susceptible peppers (Oostenbrink, 1966; Proite et al., 2008). Egg masses picked from the root systems were submerged in 2% NaOCl for approximately 5 minutes, and then counted under the microscope (Hussey & Barker, 1973). J2s were obtained from soil (100 g) using the Modified Baermann Funnel Method (Hooper, 1986) and counted under the microscope. The RF values of V3 isolate in peppers were calculated as mentioned above.

Data analyses

The differences between the egg mass index, gall index, eggs and galls per gram of fresh roots and RF values were analyzed statistically. Initially, the log₁₀(x+1) transformation was performed for data. Then, an analysis of variance (ANOVA) was applied, and the differences between the means were compared with Tukey multiple comparison test (p≤0.05) using the SAS (SAS Institute Inc., Cary, NC). The effects of plant variety, nematode isolate, and plant variety*nematode isolate interaction on the number of egg masses and galls per gram of root were analysed by two-way ANOVA. Standard errors are given with the means in the tables.

Results and Discussion

All *Mi-1.2* virulent *M. incognita* isolates used in the tests caused galls, multiplied and produced egg masses on resistant tomato Seval F1. Thus, it was confirmed that all isolates were *Mi-1.2* virulent. However, the number of egg masses and galls on resistant tomatoes caused by some isolates was significantly different (p≤0.05). V6 isolates caused the most egg masses and galls. Results showed that the virulence levels of isolates were different (Table 1).

Table 1. Mean of egg masses per g root, egg masses index, galls per g root and gall index caused by *Mi-1.2* virulent *Meloidogyne incognita* isolates on resistant Seval F1 tomato cultivar

Isolate	Egg masses/g root		Egg mass index		Galls/g root		Gall index	
V3	62.51 ± 7.43	AB	4.9 ± 0.10	A	75.37 ± 4.54	AB	5.0 ± 0.00	A
V6	76.02 ± 9.41	A	4.9 ± 0.10	A	85.78 ± 9.51	A	5.0 ± 0.00	A
V13	41.21 ± 8.45	BC	4.1 ± 0.20	A	71.67 ± 8.45	ABC	4.8 ± 0.15	A
V15	49.27 ± 5.22	AB	4.6 ± 0.16	A	51.69 ± 3.82	C	4.8 ± 0.13	A
V18	54.17 ± 6.83	AB	4.9 ± 0.15	A	60.41 ± 5.78	ABC	4.9 ± 0.10	A
V30	20.88 ± 2.00	C	4.3 ± 0.22	A	52.79 ± 4.32	BC	5.0 ± 0.00	A

Values within a column followed by a different uppercase letter are significantly different (p≤0,05) according to Tukey's multiple range test. ± indicates standard error. Untransformed data is presented in the table, all statistical analyses were conducted on the log₁₀(x+1) transformed data.

All *Mi-1.2* virulent *M. incognita* isolates caused galls, multiplied, and reproduced egg masses on susceptible pepper cultivar Safran F1. However, the number of egg masses and galls in susceptible pepper caused by some isolates were significantly different from each other (p≤0.05). V3 isolate caused the least egg masses and galls. Results showed that the pathogenicity capacity of *Mi-1.2* virulent *M. incognita* isolates was different on susceptible pepper cultivar Safran F1 (Table 2).

Table 2. Mean of egg masses per g root, egg masses index, galls per g root and gall index caused by *Mi-1.2* virulent *Meloidogyne incognita* isolates on Safran F1 pepper cultivar

Isolate	Egg masses/g root	Egg mass index	Galls/g root	Gall index
V3	10.39 ± 1.99 B	3.4 ± 0.17 A	13.91 ± 2.25 C	3.8 ± 0.15 B
V6	41.80 ± 7.34 A	4.5 ± 0.27 A	45.17 ± 5.66 A	4.9 ± 0.12 A
V13	33.19 ± 7.47 A	4.1 ± 0.31 A	47.38 ± 4.89 A	4.7 ± 0.17 A
V15	18.51 ± 3.77 AB	4.1 ± 0.23 A	19.28 ± 3.53 C	4.1 ± 0.20 AB
V18	17.55 ± 2.87 AB	3.9 ± 0.18 A	19.64 ± 2.38 BC	3.9 ± 0.10 B
V30	22.70 ± 6.15 AB	4.0 ± 0.29 A	35.00 ± 5.09 AB	4.3 ± 0.23 AB

Values within a column followed by a different uppercase letter are significantly different ($P \leq 0,05$) according to Tukey's multiple range test. \pm indicates standard error. Untransformed data is presented in the table, all statistical analyses were conducted on the $\log_{10}(x+1)$ transformed data.

All *Mi-1.2* virulent *M. incognita* isolates caused galls and reproduced egg masses on resistant pepper cultivar MT-01 F1. However, the number of egg masses and galls in resistant pepper caused by some isolates were significantly different from each other ($p \leq 0,05$). V3 isolate caused the least egg masses and galls. Results showed that the virulence levels of isolates were different (Table 3).

Table 3. Mean of egg masses per g root, egg masses index, galls per g root and gall index caused by *Mi-1.2* virulent *Meloidogyne incognita* isolates on MT-01 F1 pepper cultivar

Isolate	Egg masses/g root	Egg mass index	Galls/g root	Gall index
V3	0.35 ± 0.17 C	0.6 ± 0.22 C	0.35 ± 0.17 C	0.6 ± 0.22 B
V6	88.58 ± 16.99 A	5.0 ± 0.00 A	80.44 ± 13.24 A	5.0 ± 0.00 A
V13	56.51 ± 10.51 A	4.8 ± 0.15 A	62.37 ± 5.44 A	4.9 ± 0.11 A
V15	20.72 ± 3.14 B	3.8 ± 0,13 B	25.26 ± 3.31 B	4.0 ± 0.15 A
V18	15.33 ± 2.68 B	3.6 ± 0.16 B	21.42 ± 3.81 B	3.7 ± 0.15 A
V30	12.25 ± 0.90 B	4.0 ± 0.00 AB	23.87 ± 2.09 B	4.5 ± 0.17 A

Values within a column followed by a different uppercase letter are significantly different ($p \leq 0,05$) according to Tukey's multiple range test. \pm indicates standard error. Untransformed data is presented in the table, all statistical analyses were conducted on the $\log_{10}(x+1)$ transformed data.

In the conducted study, it was determined that *Mi-1.2* virulent *M. incognita* isolates showed different virulence on susceptible and resistant pepper carrying *Me1* cultivars. Differences in egg mass and gall numbers were found on susceptible and resistant pepper cultivars. *Mi-1.2* natural virulent V3 caused numerically fewer egg masses and galls on the susceptible pepper Safran F1 than the other isolates (Table 2). V3 isolate had about 75% fewer egg masses/g root than V6, which had the highest egg masses/g root value. Similarly, it produced few egg masses and galls on the resistant pepper cultivar MT-01 F1. It was observed that the gall/g root value of V3 was 83.7% of the value of V18, which is the closest to V3 (Table 3).

Since the *Mi-1.2* virulent *M. incognita* V3 isolate could not reproduce as much as the other isolates on the resistant pepper cultivar, the reproduction factor (RF) was calculated. For this purpose, the resistant pepper cultivar MT-01 F1 and susceptible pepper cultivar Safran F1 were retested, and the RF of the isolate on both varieties were compared. The RF of the V3 isolate was 13.32 ± 4.4 ($RF > 1$) on the susceptible pepper cultivar Safran F1, and 0.19 ± 0.08 ($RF < 1$) on the resistant pepper cultivar MT-01 F1. These results confirmed that the *Mi-1.2* virulent V3 isolate of *M. incognita* did not grow on the resistant pepper cultivar MT-01 F1 and the cultivar was resistant to V3 isolate.

In this study, plant variety (F value= 77.2; df=2; p<0.0001), nematode isolate (F value= 28.4; df=5; p<0.0001), and plant variety-nematode isolate interaction (F value=5.8; df= 10; p<0.0001) were found to have statistically significant effects on the number of galls formed per gram of root. Similarly, plant variety (F value= 26.3; df=2; p<0.0001), nematode isolate (F value= 21.9; df=5; p<0.0001), and plant variety-nematode isolate interaction (F value=6.2; df=10; p<0.0001) were found to have statistically significant effects on the number of egg masses formed per gram of root.

The proliferation of *Mi-1.2* virulent populations has become a major challenge in tomato production areas (Verdejo-Lucas et al., 2012). This situation underscores the importance of investigating the responses of different plants to *Mi-1.2* virulent isolates. The response of different resistance genes in pepper (Castagnone-Sereno et al., 2001; Djian-Caporalino et al., 2011), cucurbit rootstock *Cucumis metuliferus* E. Mey. ex Schrad. (Cucurbitales: Cucurbitaceae) (Exposito et al., 2018) and eggplant rootstock *Solanum torvum* Sw. (Solanales: Solanaceae) (Öçal et al., 2018), against *Mi-1* virulent isolates was investigated. The results show that the reproductive potential of virulent populations in different plants might vary.

The ability of five isolates tested in this study to grow and form galls in peppers carrying the *Me1* resistance gene may limit the potential of using resistant pepper varieties carrying the *Me1* gene instead of tomatoes in crop rotation in areas infected with *Mi-1.2* virulent *M. incognita*. On the other hand, further studies are needed regarding host-nematode interaction to determine why an isolate (V3) used in the study, unlike other isolates, produced many galls and egg masses in susceptible pepper plants but could not reproduce in resistant plants. In addition, testing the V3 isolate on pepper cultivars carrying the *Me1* gene with different genetic backgrounds might contribute to understanding host-nematode interaction. Previous study has indicated that a plant variety's response to different nematode isolates can differ statistically (Özalp et al., 2024). It is also anticipated that a nematode isolate may exhibit varying feeding and reproduction rates on different varieties (Nas et al., 2022). Interestingly, in this study, one of the *Mi-1* virulent isolates reproduced very limitedly in the resistant variety carrying the *Mi-1* gene, while other virulent isolates were able to form a high number of egg masses.

Castagnone-Sereno et al. (1992) reported that *Mi-1.2* virulent RKNs lost their ability to reproduce on susceptible pepper varieties. Similarly, Castagnone-Sereno et al. (2001) observed that the reproduction of *Mi-1.2* virulent populations decreased on susceptible peppers with few exceptions. Tzortzakakis et al. (1999) investigated the responses of *M. javanica* (*Mi-1.2* virulent and avirulent) and *M. incognita* (*Mi-1.2* avirulent) populations on resistant tomato and susceptible pepper varieties. They stated that *M. javanica* populations did not produce on susceptible pepper varieties, but all *M. incognita* populations produced. Tzortzakakis & Blok (2007) examined the response of *Mi-1.2* (a)virulent isolates of *M. incognita* in 10 different pepper cultivars. They found that the avirulent isolate reproduced on all plants, while *Mi-1.2* virulent isolate could not reproduce in any of them. In another study, it was determined that while none of the four *Mi-1.2* virulent *M. javanica* populations developed on susceptible pepper cultivars, one of the two *Mi-1.2* virulent *M. incognita* populations reproduced while the other did not (Tzortzakakis et al., 2016). Similarly, in this study, it was determined that five *Mi-1.2* virulent isolates were able to grow in susceptible pepper. However, V3 isolate grew less on susceptible pepper than other isolates.

Previous studies have reported that the *Me1* gene confers resistance to *Mi-1.2* virulent populations (Castagnone-Sereno et al., 1992, 1996, 2001). On the other hand, in this study, pepper bearing *Me1* gene showed resistance to only one (V3) of six *Mi-1.2* virulent *M. incognita* isolates. Özalp et al. (2024) investigated the response of *Mi-1.2* virulent and avirulent *Meloidogyne* spp. on eight pepper genotypes. As a result of testing, they reported that *Mi-1.2* virulent *M. javanica* isolates did not cause egg masses or galls on roots. However, the same study reported that *Mi-1.2* virulent *M. incognita* caused many egg masses and galls on susceptible peppers. In contrast, no egg masses and galls were observed on resistant peppers carrying *N* gene. In the present study, similar results were obtained on susceptible pepper cultivar. However, the

response of *N* gene to the *Mi-1.2* virulent isolates and the response of the *Me1* investigated in this study to the *Mi-1.2* virulent isolates were not similar. These results showed that there is variability in infection ability and pathogenicity ability within the population of *Mi-1.2* virulent *M. incognita* isolates, depending on the plant variety and whether the plant carries a resistance gene.

Knowing how the resistance provided by the *Me1* gene in pepper will perform against *Mi-1.2* virulent RKNs is particularly important for the effectiveness of the resistance, durability of the resistance, and sustainability of crop rotation. In this study, five isolates were found to cause egg masses and galls, while one isolate did not multiply on the resistant pepper cultivar bearing *Me1* gene. Resistant and susceptible pepper cultivars exhibited different responses against *Mi-1.2* virulent *M. incognita* isolates. It should be investigated whether inoculum density influences the multiplication of *Mi-1.2* virulent isolates on the peppers bearing *Me1* gene in the future studies. The results obtained might contribute to preventing product loss due to RKNs in areas with intensive vegetable production or taking precautions against virulent populations. Since the response of nematodes can vary in resistant plants with different genetic backgrounds, it will be important to investigate the response of pepper varieties with different genotypes carrying the *Me1* gene to *Mi-1* virulent RKNs in future studies for the effective use and performance of resistant varieties.

Acknowledgements

The authors would like to thank Multi Tohum Tar. San. Tic. A.Ş. and Yüksel Tohum Tarım Sanayi Ticaret A.Ş. for providing vegetable seeds.

References

- Berthou, F., A. Ba-Diallo, L. De Maeyer & G. De Guiran, 1989. Caractérisation chez les nématodes *Meloidogyne* Goeldi (Tylenchida) de types virulents vis-à-vis du gène *Mi* de la tomate dans deux zones maraîchères au Sénégal. *Agronomie*, 9: 877-884 (in French with abstract in English).
- Carneiro, R. M., V. R. Correa, M. R. A. Almeida, A. C. M. Gomes, A. M. Deimi, P. Castagnone-Sereno & G. Karssen, 2014. *Meloidogyne luci* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode parasitising different crops in Brazil, Chile and Iran. *Nematology*, 16 (3): 289-301.
- Castagnone-Sereno, P., M. Bongiovanni, A. Palloix & A. Dalmasso, 1996. Selection for *Meloidogyne incognita* virulence against resistance genes from tomato and pepper and specificity of the virulence/resistance determinants. *European Journal of Plant Pathology*, 102 (6): 585-590.
- Castagnone-Sereno, P., M. Bongiovanni & A. Dalmasso, 1992. Differential expression of root-knot nematode resistance genes in tomato and pepper: evidence with *Meloidogyne incognita* virulent and avirulent near-isogenic lineages. *Annals of Applied Biology*, 120 (3): 487-492.
- Castagnone-Sereno, P., M. Bongiovanni & C. Djian-Caporalino, 2001. New data on the specificity of the root-knot nematode resistance genes *Me1* and *Me3* in pepper. *Plant Breeding*, 120 (5): 429-433.
- Changkwian, A., J. Venkatesh, J. H. Lee, J. W. Han, J. K. Kwon, M. I. Siddique, A. M. Solomon, G. J. Choi, E. Kim, Y. Seo, Y. H. Kim & B. C. Kang, 2019. Physical localization of the root-knot nematode (*Meloidogyne incognita*) resistance locus *Me7* in pepper (*Capsicum annuum*). *Frontiers in Plant Science*, 10: 886 (1-15).
- Devran, Z. & M. A. Söğüt, 2010. Occurrence of virulent root-knot nematode populations on tomatoes bearing the *Mi* gene in protected vegetable-growing areas of Turkey. *Phytoparasitica*, 38 (3): 245-251.
- Djian-Caporalino, C., A. Fazari, M. J. Argue, T. Vernie, C. Vandecasteele, I. Faure, G. Brunoud, L. Pijarowski, A. Palloix & V. Lefebvre, 2007. Root-knot nematode (*Meloidogyne* spp.) *Me* resistance genes in pepper (*Capsicum annuum* L.) are clustered on the P9 chromosome. *Theoretical and Applied Genetics*, 114 (3): 473-486.
- Djian-Caporalino, C., L. Pijarowski, A. Fazari, M. Samson, L. Gaveau, C. O'byrne, V. Lefebvre, C. Caranta, A. Palloix & P. Abad, 2001. High-resolution genetic mapping of the pepper (*Capsicum annuum* L.) resistance loci *Me3* and *Me4* conferring heat-stable resistance to root-knot nematodes (*Meloidogyne* spp.). *Theoretical and Applied Genetics*, 103 (4): 592-600.

- Djian-Caporalino, C., L. Pijarowski, A. Januel, V. Lefebvre, A. Daubeze, A. Palloix, A. Dalmasso & P. Abad, 1999. Spectrum of resistance to root-knot nematodes and inheritance of heat-stable resistance in pepper (*Capsicum annuum* L.). *Theoretical and Applied Genetics*, 99 (3-4): 496-502.
- Djian-Caporalino, C., S. Molinari, A. Palloix, A. Ciancio, A. Fazari, N. Marteu, N. Ris & P. Castagnone-Sereno, 2011. The reproductive potential of the root-knot nematode *Meloidogyne incognita* is affected by selection for virulence against major resistance genes from tomato and pepper. *European Journal of Plant Pathology*, 131 (3): 431-440.
- Dropkin, V., 1969. The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne*: reversal by temperature. *Phytopathology*, 59: 1632-1637.
- Eddaoudi, M., M. Mammati & H. Rammah, 1997. Identification of resistance breaking populations of *Meloidogyne* on tomatoes in Morocco and their effect on new sources of resistance. *Fundamental and Applied Nematology*, 20 (3): 285-289.
- Expósito, A., M. Munera, A. Giné, M. López-Gómez, A. Cáceres, B. Picó, C. Gisbert & F. J. Sorribas, 2018. *Cucumis metuliferus* is resistant to root-knot nematode *Mi1.2* gene (a) virulent isolates and a promising melon rootstock. *Plant Pathology*, 67 (5): 1161-1167.
- Hajihassani, A., W. B. Rutter & X. Luo, 2019. Resistant pepper carrying *N*, *Me1*, and *Me3* have different effects on penetration and reproduction of four major *Meloidogyne* species. *Journal of Nematology*, 51 (1): 1-9.
- Hartman, K. M. & J. N. Sasser, 1985. "Identification of *Meloidogyne* Species on The Basis of Different Host Test and Perineal Pattern Morphology, 69-77". In: *An Advanced Treatise on Meloidogyne. Methodology*, Vol 2. (Eds. K. R. Barker, C. C. Carter & J. N. Sasser). North Carolina State University Graphics, Raleigh, 223 pp.
- Hooper, D. J., 1986. "Handling, Fixing, Staining and Mounting Nematodes, 59-80". In: *Laboratory Methods for Work with Plant and Soil Nematodes* (Ed. J. F. Southey). Her Majesty's Stationery Office, London, 202 pp.
- Hussey, R. S. & K. R. Barker, 1973. Comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter*, 57 (12): 1025-1028.
- Iberkleid, I., R. Ozalvo, L. Feldman, M. Elbaz, B. Patricia & S. B. Horowitz, 2014. Responses of tomato genotypes to avirulent and *Mi*-virulent *Meloidogyne javanica* isolates occurring in Israel. *Phytopathology*, 104 (5): 484-496.
- Jacquet, M., M. Bongiovanni, M. Martinez, P. Verschave, E. Wajnberg & P. Castagnone-Sereno, 2005. Variation in resistance to the root-knot nematode *Meloidogyne incognita* in tomato genotypes bearing the *Mi* gene. *Plant Pathology*, 54 (2): 93-99.
- Kaloshian, I., V. Williamson, G. Miyao, D. Lawn & B. Westerdahl, 1996. "Resistance-breaking" nematodes identified in California tomatoes. *California Agriculture*, 50 (6): 8-19.
- Lopez-Perez, J. A., M. Le Strange, I. Kaloshian & A. T. Ploeg, 2006. Differential response of *Mi* gene-resistant tomato rootstocks to root-knot nematodes (*Meloidogyne incognita*). *Crop Protection*, 25 (4): 382-388.
- Mıstanođlu, İ., 2020. *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949'nın Avirulent ve *Mi-1* Virulent Populasyonlari Arasındaki DNA Dizilim Farklılıklarının Yeni Nesil Dizileme Yöntemiyle Belirlenmesi, Akdeniz Üniversitesi, (Unpublished) PhD Thesis, Antalya, 68 s (in Turkish with abstract in English).
- Mıstanođlu, İ., T. Özalp & Z. Devran, 2020. The efficacy of molecular markers associated with virulence in root-knot nematodes. *Nematology*, 22 (2): 147-154.
- Narabu, T. & Y. Momota, 1992. Occurrence and analysis of resistance breaking populations of *Meloidogyne incognita* on tomato cultivars. *Proceedings of the Kanto-Tosan Plant Protection Society*, 39: 297-299.
- Nas, Y., T. Özalp & Z. Devran, 2022. Screening of Urfa pepper landraces for resistance to *Meloidogyne incognita*. *Journal of Plant Diseases and Protection*, 130 (1): 77-83.
- Öçal, S., T. Özalp & Z. Devran, 2018. Reaction of wild eggplant *Solanum torvum* to different species of root-knot nematodes from Turkey. *Journal of Plant Disease and Protection*, 125 (6): 577-580.
- Oostenbrink, M., 1966. Major characteristics of the relation between nematodes and plants. *Mededelingen Rijksfaculteit Landbouwwetenschappen Gent*, 66 (4): 3-46.
- Ornat, C., S. Verdejo-Lucas & F. Sorribas, 2001. A population of *Meloidogyne javanica* in Spain virulent to the *Mi* resistance gene in tomato. *Plant Disease*, 85 (3): 271-276.
- Özalp, T. & Z. Devran, 2018. Response of tomato plants carrying *Mi-1* gene to *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 under high soil temperatures. *Turkish Journal of Entomology*, 42 (4): 313-322.

- Özalp, T., E. S. Çelik, E. Özkaynak & Z. Devran, 2024. Host suitability of pepper cultivars to (a) virulent root-knot nematodes isolates. Turkish Journal of Entomology, 48 (1): 35-40.
- Proite, K.; R. Carneiro, R. Falcão, A. Gomes, S. Leal-Bertioli, P. Guimarães & D. Bertioli, 2008. Post-infection development and histopathology of *Meloidogyne arenaria* race 1 on *Arachis* spp. Plant Pathology, 57 (5): 974-980.
- Riggs, R. D. & N. N. Winstead, 1959. Studies on resistance in tomato to root-knot nematodes and on occurrence of pathogenic biotypes. Phytopathology, 49 (11): 716-724.
- Roberts, P. A., 1990. "Resistance in Nematodes: Definition, Concepts and Consequences, 1-15". In: Methods for Evaluating Plant Species for Resistance to Plant Parasitic Nematodes (Ed. J. L. Starr). Society of Nematologists, Hyattsville, 87 pp.
- Sargın, S. & Z. Devran, 2021. Degree of resistance of *Solanum torvum* cultivars to *Mi-1.2*-virulent and avirulent isolates of *Meloidogyne incognita*, *Meloidogyne javanica*, and *Meloidogyne luci*. Journal of Nematology, 53 (1): 1-7.
- Sikora, R. A., K. Sitaramaiah & R. S. Singh, 1973. Reaction of root-knot nematode-resistant tomato cultivars to *Meloidogyne javanica* in India. Plant Disease Reporter, 57 (2): 141-143.
- Silva, R., B. Lima, F. Peixoto, J. Gondim & B. Miranda, 2019. Supplanting resistance of the *Mi* gene by root-knot nematode in industrial tomato in the Cerrado in Goiás state of Brazil. Ciência Rural, 49 (9): e20180784.
- Talavera-Rubia, M., M. D. Vela-Delgado & S. Verdejo-Lucas, 2022. A cost-benefit analysis of soil disinfestation methods against root-knot nematodes in Mediterranean intensive horticulture. Plants, 11 (20): 1-11.
- Tzortzakakis, E. & V. Blok, 2007. Differentiation in two populations of *Meloidogyne incognita* from Greece in relation to reproduction on resistant tomato and pepper. Journal of Plant Disease and Protection, 114 (6): 276-277.
- Tzortzakakis, E. A., M. A. Adam, V. C. Bol, C. Paraskevopoulos & K. Bourtzis, 2005. Occurrence of resistance-breaking populations of root-knot nematodes on tomato in Greece. European Journal of Plant Pathology, 113 (1): 101-105.
- Tzortzakakis, E. A., V. C. Blok, M. S. Phillips & D. L. Trudgill, 1999. Variation in root-knot nematode (*Meloidogyne* spp.) in Crete in relation to control with resistant tomato and pepper. Nematology, 1 (5): 499-506.
- Tzortzakakis, E., M. C. V. Dos Santos & I. Conceição, 2016. An update on the occurrence of resistance-breaking populations of root-knot nematodes (*Meloidogyne* spp.) on resistant tomato in Greece with six new records from Crete. Hellenic Plant Protection Journal, 9 (2): 60-65.
- Verdejo-Lucas, S., M. Talavera & M. F. Andrés, 2012. Virulence response to the *Mi-1* gene of *Meloidogyne* populations from tomato in greenhouses. Crop Protection, 39: 97-105.
- Williamson, V. M. & R. S. Hussey, 1996. Nematode pathogenesis and resistance in plants. The Plant Cell, 8 (10): 1735-1745.