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# Detection of Root-Knot Nematode Species and Races in Kahramanmaraş Province, Türkiye

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## ABSTRACT
Root-knot nematodes (*Meloidogyne* spp.) are organisms that spread over large areas and cause economic damage to vegetables. In this study, root-knot nematode populations obtained from vegetable growing areas of Kahramanmaraş province were identified. Overall, 132 root samples were taken from the vegetable crop fields. Root-knot nematode was detected in 25 of the collected samples and their diagnosis was determined based on biochemical (esterase isoenzyme phenotype), perineal pattern, and molecular methods. The race determination of root-knot nematodes was made according to the North Carolina Differential Host Test. Results showed that while *Meloidogyne incognita* was detected in Andırın, Onikişubat, Dulkadiroğlu, Türkoğlu, and Ekinözü districts of Kahramanmaraş, both *M. incognita* and *M. javanica* were found in Beyoğlu of Türkoğlu. This is the first report of *M. javanica* infection in Kahramanmaraş. Races of root-knot nematodes were determined as *M. incognita* race 1, race 2 and *M. javanica* race 2.

**Plant Protection**

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# Kahramanmaraş İlindeki Kök-Ur Nematodu Tür ve Irklarının Belirlenmesi, Türkiye

## ÖZET
Bitki paraziti kök-ur nematodları (*Meloidogyne* spp.) geniş alanlara yayılan ve sebzelerde ekonomik zararlara neden olan canlılardır. Bu çalışmada, Kahramanmaraş ilindeki sebze yetiştirme alanlarından elde edilen kök-ur nematodlarının teşhisi yapılmıştır. Genel olarak, sebze alanlarından 132 adet kök örneği alınmıştır. Toplanan örneklerden 25 adetinde kök-ur nematodu tespit edilmiş ve teşhisleri biyokimyasal (esteraz izoenzim fenotipi), perineal kesit ve moleküler yöntemlere göre belirlenmiştir. Kök-ur nematodlarının ırk tespiti Kuzey Karolina Konukçu Testi'ne göre yapılmıştır. Çalışma sonunda, Kahramanmaraş'ın Andırın, Onikişubat, Dulkadiroğlu, Türkoğlu, Ekinözü ilçelerinde *Meloidogyne incognita*, Türkoğlu Beyoğlu'nda ise hem *M. incognita* hem de *M. javanica* tespit edilmiştir. Kahramanmaraş ilinde *M. javanica* ilk kez bulunmuştur. Kök-ur nematodlarının ırkları *M. incognita* ırk 1, ırk 2 ve *M. javanica* ırk 2 olarak belirlenmiştir.

**Bitki Koruma**

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Irk

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## INTRODUCTION
Among several biotic stresses, root-knot nematodes (RKN) are a major threat to vegetable production. Tomato, pepper and eggplant are some of their important host plants. Eggplant and tomato are known to be extremely susceptible to root-knot nematodes infection, causing severe damage that leads to great losses (Gowda et al. 2019; Tapia-Vázquez et al. 2022; Shaaban et al. 2023). Generally, root-knot nematodes are easily spread through vegetable areas (Collange et al. 2011; Rao et al. 2015). Second-stage juveniles (J2) cause the main damage to root cells of plants. Continuously sucking plant tissue through their styles gives rise to plants nutrition and element deficits.

Thus, it causes adverse effects on plant growth. RKN infections characteristically lead to the formation of galls on roots.

Plants that are infected by RKN lead to crop reduction, stunted growth, yellowing, and wilting, and become more sensitive towards bacteria and fungi in plants (Wang et al. 2013). The abundance of these organisms can affect the survival of the plants. Thus when they are present in high amounts of plant roots, they may dry and eventually the plant can die (Thorne, 1961).

There are some ways of controlling root-knot nematodes, including soil solarization, crop rotation, chemical control, plant resistance, and using other plant extracts or essential oils as nematicides (Young, 1992; Roberts, 1992; Sijmons et al.1994; Tzortzakakis et al. 1999; Tytgat et al. 2000). In order to efficiently control the root-knot nematodes, diagnosis of species and detection of race methods have great importance. RKN species are mostly identified based on morphologic characters (female perineal patterns) (Eisenback & Triantaphyllou, 1991), isozyme phenotypes (Esbenshade & Triantaphyllou, 1990) and molecular techniques (Powers & Harris, 1993; Powers et al. 1997; Zijlstra et al. 1995; Zijlstra et al. 2000; Adam et al. 2007).

Biochemical and molecular techniques currently improve the accuracy of RKN species identification (Resquin-Romero et al. 2023). Gerič Stare et al. (2018, 2019) reported that the best way to distinguish root-knot nematode is to use the esterase enzyme phenotype. Esterase phenotyping has been proven to be species-specific in many cases for *Meloidogyne* species (Carneiro et al. 2001; 2008). Nevertheless, using this technique is limited to the stage of adult females, but females are not always suitable for analysis due to the state of root decomposition (Salgado et al. 2015). Specific sequence characterized amplified region (SCAR) markers have been successfully developed to diagnose the dominant tropical RKNs associated with important crops such as tomato, coffee, guava, and grapevine; these nematodes include *Meloidogyne javanica*, *M. arenaria* (Zijlstra et al. 2000), *M. incognita* (Randig et al. 2002), *M. paranaensis*, *M. exigua* (Randing et al. 2002), *M. enterolobii* (Tigano et al. 2010), *M. arabicida*, *M. izalcoensis* (Correa et al. 2013) and *M. ethiopica* (Correa et al. 2014). Because of providing a faster diagnosis possibility, molecular techniques are used in the identification of root-knot nematodes. Proper and precise analysis of plant parasitic nematodes is critical for crop protection (Devran & Söğüt, 2011; Adzitey et al. 2013).

This study aimed to diagnose root-knot nematode species and races collected from vegetable growing areas of Kahramanmaraş using perineal patterns, biochemical (esterase isozyme phenotypes), and molecular techniques.

## MATERIAL and METHOD

### Collection of galled root samples

Surveys were conducted in August and September of 2014 and 2015 in 11 districts (Afşin, Andırın, Çağlayancerit, Dulkadiroglu, Elbistan, Ekinözü, Göksun, Nurhak, Onikisubat, Pazarcık and Türkoğlu) of Kahramanmaraş province. Root-knot nematode was found in only 5 districts (Andırın, Dulkadiroğlu, Ekinözü, Onikişubat and Türkoğlu) of the 11 districts surveyed. Overall 132 roots of eggplant (*Solanum melongena*), tomato (*Lycopersicon esculentum*) and pepper (*Capsicum annuum*) were checked and the 25 samples having galls were collected, placed into polyethylene bags labelled with necessary information including coordinates and, stored at refrigerator at +4 °C until use.

### Pure cultures of nematodes

In order to make an accurate diagnosis pure cultures are needed. Egg masses of 25 populations were picked up from roots with the help of a needle for pure culture. Tomato seedlings (Falcon) were transplanted into 250-mL plastic pots filled with sterilized sandy loam soil and sand (ratio of 2:1) at the fourth true leaf stage and inoculated with a single egg mass of each population. Female and J2 were obtained 65 days after inoculation.

### Species identification

Identification of nematodes was done with biochemical (esterase isozyme phenotypes), perineal pattern, and molecular methods.

### Identification of species with the biochemical method

The females obtained pure cultures were crushed and homogenized individually in 5 µl of extraction buffer and 5 µl deionized water in an eppendorf tube. Electrophoresis process was carried out in a discontinuous buffer system with 8% acrylamide running gel, pH 8.8, and 4% acrylamide stacking gel, pH 6.8 in a Bio-Rad mini-PROTEIN II (Bio-Rad, Singapore).

Then, the homogenized sample was loaded carefully into the well in the stacking gel. The first and last wells were loaded with sample of *Meloidogyne javanica* as a reference. The voltage was maintained at 80 volts for the first 13 minutes and increased to 200 volts for the remaining 45 minutes of running period. Following electrophoresis, the gels were removed and put into a staining solution for 45 minutes in the dark. After this period samples were washed with deionized water and esterase phenotype bands were compared to diagnosis according to Esbenshade & Triantaphyllou (1985).

### Morphological characterization

Roots were washed with tap water, and cleared from residues and each female separated with help of a

scalpel. Adult females were extracted from each root system under a light microscope (EUROMEX PB 416) and were placed into %45 lactic acid for 20-25 min. Perineal patterns of females were cut and mounted in a glycerol. The perineal pattern method was completed as described by Hunt & Handoo (2009).

### Molecular characterization

**DNA extraction:** DNA was extracted from egg masses of each nematode population using DNeasy Tissue Blood Kit (QIAGEN, Hilden, Germany) according to instructions of the manufacturer. DNA samples were kept at -20 ºC until used.

**PCR conditions:** DNA amplifications were conducted with species-specific primers listed in Table 1. PCR amplifications were performed in a DNA thermal cycler with the following thermal cycling program: initial cycle, 2 min at 95°C; next 40 cycles, 1 min at 94°C; 1 min at 64°C, and 1.5 min at 72 °C for primers Fjav/Rjav and 2 min at 95 °C; next 40 cycles, 1 min at 94 °C; 1 min at 54 °C, and 1.5 min at 72 °C for primers Finc/Rinc and 2 min at 95 °C; next 40 cycles, 1 min at 94 °C; 1 min at 64 °C, and 1.5 min at 72 °C for primers Inc-K14-F/Inc-K14-R. PCR amplification reactions were performed in a total volume of 40 µL containing 4 µl of 10x PCR Buffer, 1 µl of 250 µM deoxynucleoside triphosphates (dNTPs) (Favorgen Biotech Corp., Taiwan), 1µl 20 picomoles of each primer, 2 mM MgCI2, 20 ng of template DNA and 1 Unit Taq DNA Polymerase (Vivantis). PCR was run using a Techne PHC-3 (Techne, Cambridge, UK) thermal cycler. Amplified PCR products were run on 1.5% electrophoresis gel (Bio-Rad, Hercules, CA) and analyzed under UV light.

Table 1. Primers used for molecular identification of Meloidogyne incognita and M. javanica
*Çizelge 1. Meloidogyne incognita ve M. javanica'nın moleküler tespiti için kullanılan primerler*

| *Name of primer* | *Species* | *Primer sequences (5'-3')* | *DNA Fragment (bp)* | *References* |
|---|---|---|---|---|
| ***Finc*** | *M. incognita* | CTCTGCCCAATGAGCTGTCC | 1200 | Zijlstra et al. (2000) |
| ***Rinc*** | | CTCTGCCCTCACATTAAG | | |
| ***İnc-K14F*** | *M. incognita* | CCCGCTACACCCTCAACTTC | 399 | Randig et al. (2002) |
| ***İnc-K14R*** | | GGGATGTGTAAATGCTCCTG | | |
| ***Fjav*** | *M. javanica* | GGTGCGCGATTGAACTGAGC | 670 | Zijlstra et al. (2000) |
| ***Rjav*** | | CAGGCCCTTCAGTGGAACTATAC | | |

### Race determination

The North Carolina Differential Host Test was conducted in the growth chambers. Experiment conditions were 25±1 ºC and %60±10 humidity with 16 h light. Four-leaf stage seedlings of tobacco (NC 95), cotton (Delta Pine 16), tomato (Rutgers), peanut (Florunner) and pepper (California wonder) plants were transplanted into 250 ml plastic pots filled with sterilized sandy soil (%80 sand, %20 peat). The trial was designed as a randomized plots design, with four replications. RKN egg masses were obtained from pure cultures and were incubated for two days at 28 ºC based on the modified Baermann-funnel method (Hooper 1986) and then J2 counted under stereo microscope. Seedlings were inoculated with approximately 1000 J2. They were watered as needed and fertilized during the trial. Sixty-five days after nematode inoculation, plants were harvested and egg masses on roots were assessed using 0-5 scale galling index (Hartman & Sasser, 1985).

## RESULTS

Species identification of 25 populations obtained from vegetable areas infested with RKN in Kahramanmaraş province and its districts were made using the perineal pattern, biochemical and molecular methods. Two species were identified *M. incognita* (23 populations) and *M. javanica* (2 populations). While *Meloidogyne incognita* was found in eggplant (11 populations), pepper (4 populations), and tomato (10 populations) grown in Andırın, Dulkadiroğlu, Ekinözü, Onikişubat and Türkoğlu districts, *M. javanica* was detected in tomato and eggplant in a location (Beyoğlu) in Türkoğlu district (Table 2). The morphological species diagnosis indicated that the perineal shape of *M. incognita* was oval-round, the dorsal arch was angular and high, and the striae were wavy. The perineal pattern of *M. javanica* was typical with a rounded low dorsal arch, smooth striae, and clear parallel lateral lines (Figure 1). According to the esterase isozyme phenotypes diagnostic results, three different esterase phenotypes were detected as J3, I1 and I2 in this study (Figure 2). Two population exhibited *M. javanica*-specific phenotype J3 as used reference samples. Phenotypes I1 and I2 are species-specific for *M. incognita*. Phenotype I2 was the most prevalent esterase phenotypes and were detected in 18 populations, while phenotype I1 was in 5 populations.

Species-specific SCAR primer pairs were used for the identification of *Meloidogyne incognita* and *M. javanica*. Three pairs of primers were tested on DNA samples obtained from egg masses and resulted in consistent amplifications. *Meloidogyne incognita* species primer pairs Finc/Rinc and inc-K14-F/ inc-K14-R produced a single band of 1200 bp and a single band of 399 bp for 23 populations, respectively (Figures 3 and 4). DNA samples of these populations were no amplifications when Fjav/Rjav primer set was used.

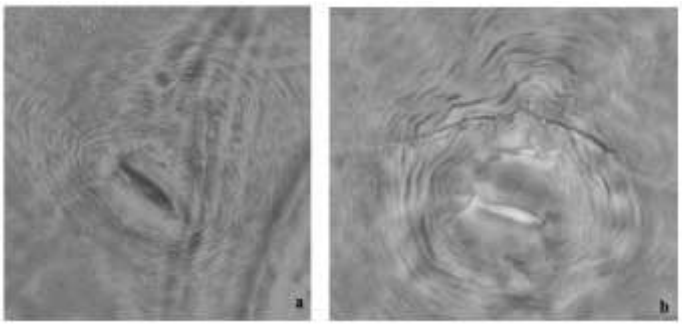

Figure 1. Perineal patterns of *Meloidogyne incognita* (a) and *Meloidogyne javanica* (b).

*Şekil 1. Meloidogyne incognita (a) ve Meloidogyne javanica (b) anal kesitleri*

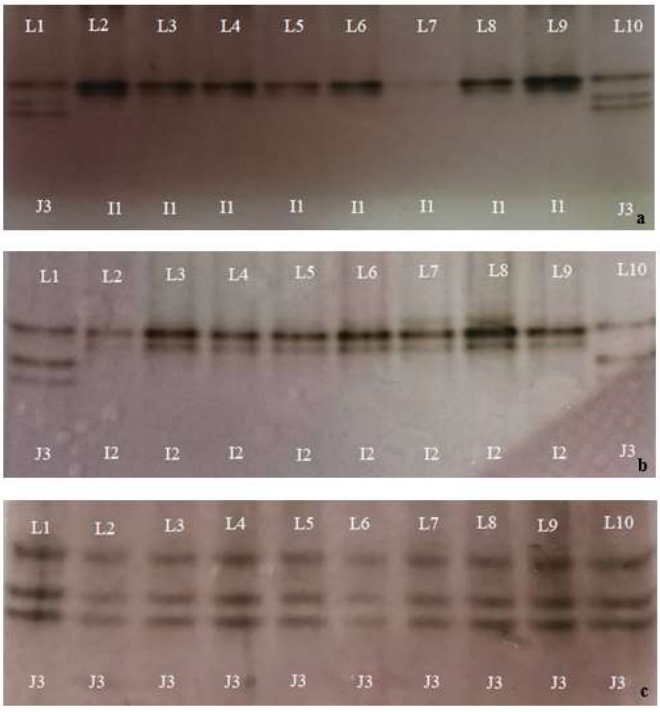


Figure 2. Esterase phenotypes results in *Meloidogyne* species a) *Meloidogyne incognita* (Est: I1) (Sample no: 56 B) b) *Meloidogyne incognita* (Est: I2) (Sample no: 49 P) c) *Meloidogyne javanica* (Est: J3) (Sample no: 53 D), Positive control (L1 and L2)

*Şekil 2. Meloidogyne türlerinin esteraz fenotip sonuçları a) Meloidogyne incognita (Est: I1) (Örnek no: 56 B) b) Meloidogyne incognita (Est: I2) (Örnek no: 49 P) c) Meloidogyne javanica (Est: J3) (Örnek no: 53 D), Pozitif kontrol (L1 and L2).*

*Meloidogyne javanica* species-specific primers Fjav/Rjav produced a band of 670 bp for two populations (Figure 5). However, the DNA of these populations was not amplified products when Finc/Rinc and inc-K14-F/ inc-K14-R primer sets were used.

In this study, race identification of root-knot nematodes was made according to the North Carolina Differential Host Test. As a result of the experiment, *M. incognita* race 1 egg mass was not found in tobacco, cotton and peanut plants but found in pepper and tomato plants. For *M. incognita* race 2 egg mass was not found in cotton and peanut plants, egg mass was observed in tobacco, pepper and tomato plants. For *M. javanica* race 2 egg mass was not found in tobacco, cotton, peanut plants, egg mass was observed in pepper and tomato plants. The races of the species were determined as *Meloidogyne incognita* race 1 (Andırın/çiçek, Döngele, Merkez; Onikisubat/Aksu; Türkoğlu/Beyoğlu), *M. incognita* race 2 (Türkoğlu/Beyoğlu and Yenipınar) and *M. javanica* race 2 (Türkoğlu/Beyoğlu) (Table 2).

## DISCUSSION and CONCLUSION

Root-knot nematode populations obtained on tomato, pepper, and eggplant plants in Kahramanmaraş and its districts were diagnosed based on perineal pattern, biochemical and molecular methods, and *Meloidogyne incognita* and *M. javanica* were determined in the study. In previous studies, it was stated that the most important and common species were *M. incognita* and *M. javanica* in Türkiye (Söğüt & Elekçioğlu, 2000; Özarslandan & Elekçioğlu, 2010). In the Eastern Mediterranean Region, *M. incognita* was found as the dominant species previously

(Söğüt & Elekçioğlu, 2000; Özarslandan & Elekçioğlu, 2010; Gürkan et al. 2019, Aslan & Elekçioğlu, 2022). Similarly, in our data, *Meloidogyne incognita* was found to be the most common RKN in Kahramanmaraş. The study by Çetintaş & Çakmak (2016), diagnosis of *Meloidogyne incognita* was made according to esterase phenotypes and perineal pattern methods and while only *Meloidogyne incognita* has been reported in Kahramanmaraş (Pazarcık, Türkoğlu, and Centre) in their study, *M. incognita* and *M. javanica* were detected in Türkoğlu/Beyoğlu in this current study. Previous studies in the Mediterranean region, Gürkan et al. (2019) the species identification study of root-knot nematodes in vegetable fields (tomato, pepper, eggplant, bean and okra) of Gaziantep and Osmaniye provinces based on biochemical and perineal methods. They identified *M. incognita*, *M. javanica* and *M. arenaria* in Gaziantep and *M. incognita*, *M. javanica*, *M. arenaria* and *M. luci* in Osmaniye. In the study of Aslan & Elekçioğlu, 2022, the diagnosis of root-knot nematodes in the greenhouse vegetable areas of the Eastern Mediterranean Region was made according to biochemical and molecular (SCAR primers) methods. *M. incognita* and *M. javanica* were detected in Mersin, Adana, and Hatay provinces.

In this study, *M. incognita* race 1 was detected in Türkoğlu, Onikişubat, and Andırın, while *M. incognita* race 2 and *M. javanica* race 2 were detected in Türkoğlu. At the end of the study, *M. incognita* race 1 and race 2, *M. javanica* race 2 in tomato and eggplant

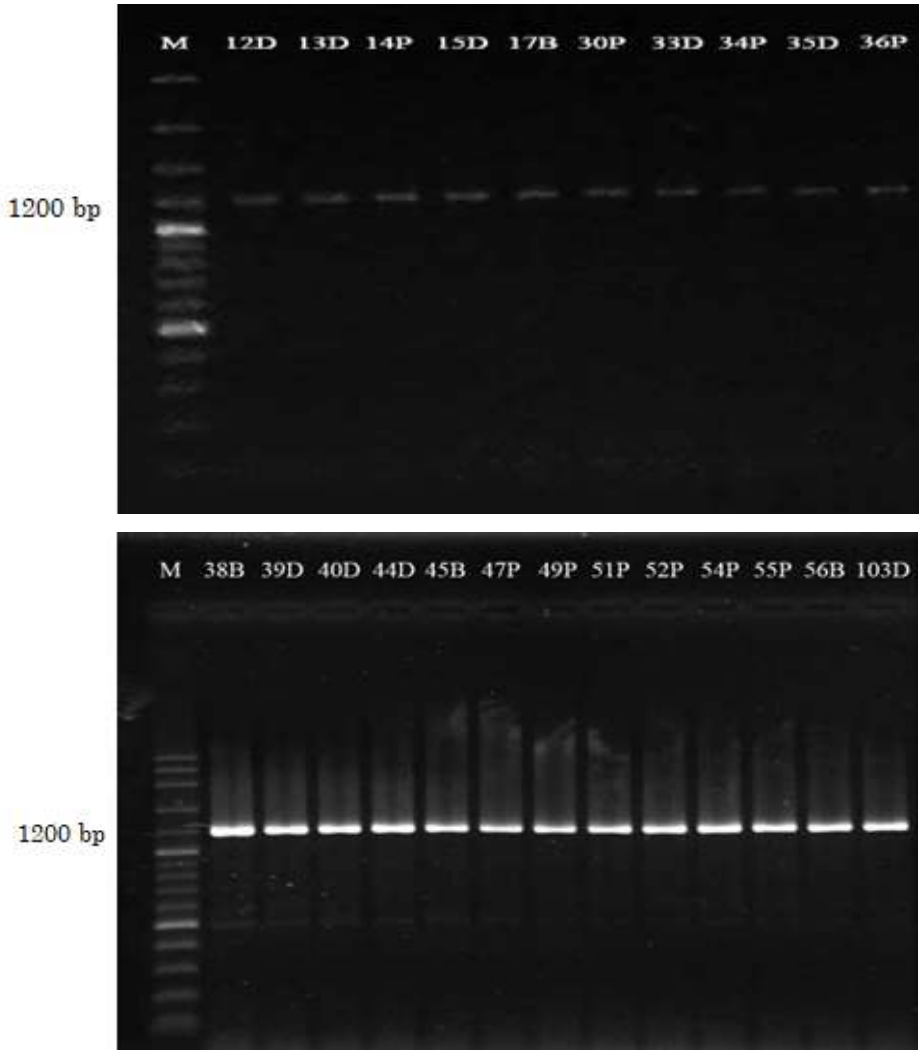


Figure 3. Amplification products with the Meloidogyne incognita Finc/Rinc SCAR primers. M: 100 bp DNA Ladder (Vivantis), Samples (12D-103D).

*Şekil 3. Meloidogyne incognita Finc/Rinc SCAR primerleri ile amplifikasyon ürünleri. M: 100 bp DNA Ladder (Vivantis), Örnekler (12D-103D).*

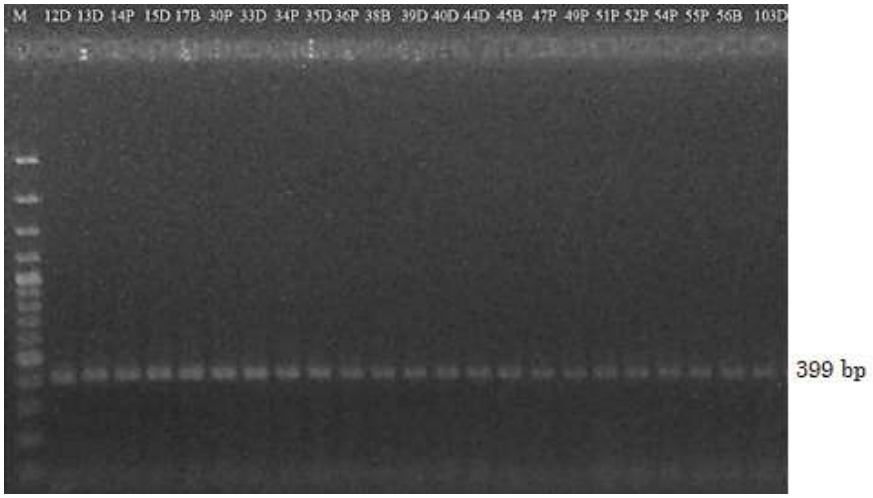


Figure 4. Amplification products with the Meloidogyne incognita Inc-K14-F/Inc-K14-R SCAR primers. M: 100 bp DNA Ladder (Vivantis), Samples (12D-103D).

*Şekil 4. Meloidogyne incognita Inc-K14-F/Inc-K14-R SCAR primerleri ile amplifikasyon ürünleri. M: 100 bp DNA Ladder (Vivantis), Örnekler (12D-103D).*

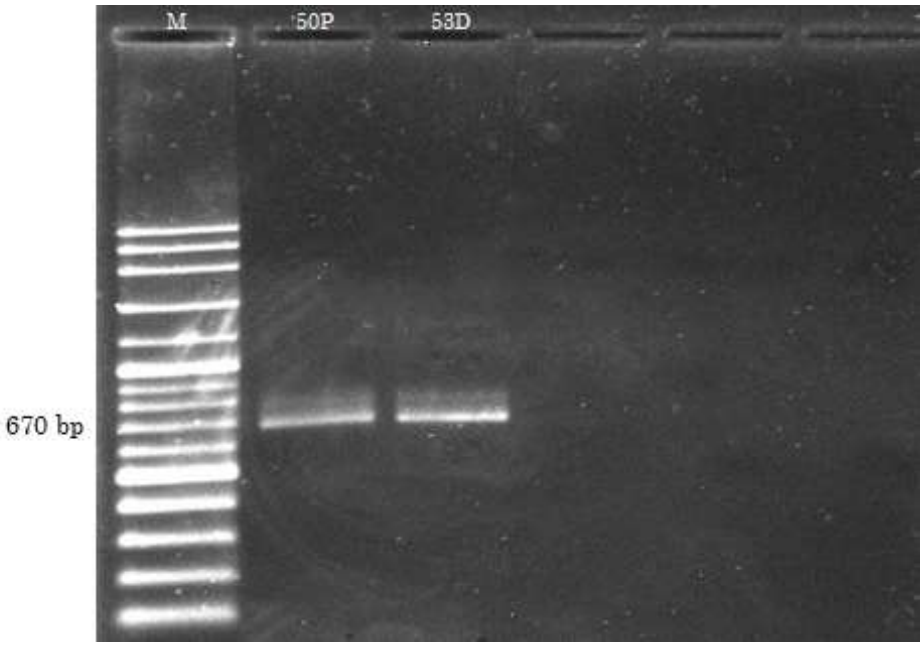


Figure 5. Amplification products with the Meloidogyne javanica Fjav/Rjav SCAR primers. M: 100 bp DNA Ladder (Vivantis), Samples (50P and 53D).

*Şekil 5. Meloidogyne javanica Fjav/Rjav SCAR primerleri ile amplifikasyon ürünleri. M: 100 bp DNA Ladder (Vivantis), Örnekler (50P ve 53D).*

*Table 2. Species and race determination in Kahramanmaraş and its districts*

Çizelge 2. Kahramanmaraş ve ilçelerindeki tür ve ırk tespiti

| *Sample Code* | *Host plant* | *District/Location* | *Latitude* | *Longitude* | *Altitude (m)* | *Species identification** | *Race identification* |
|---|---|---|---|---|---|---|---|
| *12D* | Tomato | Türkoğlu/Aydın Kavak | 37º24'37" | 36º47'25" | 696 | *M. incognita* (Est I1) | Race 1 |
| *13D* | Tomato | Türkoğlu/Aydın Kavak | 37º25'12" | 36º48'13" | 682 | *M. incognita* (Est I1) | Race 1 |
| *14P* | Eggplant | Türkoğlu/Yenipınar | 37º25'28" | 36º48'29" | 726 | *M. incognita* (Est: I2) | Race 2 |
| *15D* | Tomato | Türkoğlu/Yenipınar | 37º25'28" | 36º48'29" | 727 | *M. incognita* (Est: I2) | Race 2 |
| *17B* | Pepper | Onikişubat/Aksu | 37º32'08" | 36º55'01" | 490 | *M. incognita* (Est: I2) | Race 1 |
| *30P* | Eggplant | Dulkadiroğlu/Çiğli | 37º29'272" | 37º03'986" | 689 | *M. incognita* (Est: I2) | Race 1 |
| *33D* | Tomato | Andırın/Döngele | 37º33'847" | 36º38'447" | 609 | *M. incognita* (Est: I2) | Race 1 |
| *34P* | Eggplant | Andırın/Döngele | 37º33'663" | 36º38'442" | 652 | *M. incognita* (Est: I2) | Race 1 |
| *35D* | Tomato | Andırın/Döngele | 37º33'479" | 36º38'236" | 671 | *M. incognita* (Est: I2) | Race 1 |
| *36P* | Eggplant | Andırın/Döngele | 37º33'434" | 36º38'480" | 691 | *M. incognita* (Est: I2) | Race 1 |
| *38B* | Pepper | Andırın/Durdular | 37º33'352" | 36º38'042" | 704 | *M. incognita* (Est: I2) | Race 1 |
| *39D* | Tomato | Andırın/Centre | 37º34'217" | 36º21'523" | 995 | *M. incognita* (Est: I1) | Race 1 |
| *40D* | Tomato | Andırın/Çiçekli | 37º34'704" | 36º20'168" | 1102 | *M. incognita* (Est: I2) | Race 1 |
| *44D* | Tomato | Andırın/Centre | 37º31'760" | 36º22'240" | 644 | *M. incognita* (Est: I2) | Race 1 |
| *45B* | Pepper | Türkoğlu/Beyoğlu | 37º17'213" | 36º09'630" | 503 | *M. incognita* (Est: I2) | Race 1 |
| *47P* | Eggplant | Türkoğlu/Beyoğlu | 37º17'129" | 36º47'101" | 506 | *M. incognita* (Est: I1) | Race 1 |
| *49P* | Eggplant | Türkoğlu/Beyoğlu | 37º17'157" | 36º47'084" | 509 | *M. incognita* (Est: I2) | Race 1 |
| *50P* | Eggplant | Türkoğlu/Beyoğlu | 37º17'254" | 36º47'124" | 512 | *M. javanica* (Est: J3) | Race 2 |
| *51P* | Eggplant | Türkoğlu/Beyoğlu | 37º17'259" | 36º47'120" | 511 | *M. incognita* (Est: I2) | Race 1 |
| *52P* | Eggplant | Türkoğlu/Beyoğlu | 37º17'271" | 36º47'104" | 515 | *M. incognita* (Est: I2) | Race 1 |
| *53D* | Tomato | Türkoğlu/Beyoğlu | 37º17'442" | 36º47'337" | 503 | *M. javanica* (Est: J3) | Race 2 |
| *54P* | Eggplant | Türkoğlu/Beyoğlu | 37º17'453" | 36º47'337" | 504 | *M. incognita* (Est: I2) | Race 2 |
| *55P* | Eggplant | Türkoğlu/Beyoğlu | 37º17'440" | 36º47'332" | 507 | *M. incognita* (Est: I2) | Race 2 |
| *56B* | Pepper | Türkoğlu/Beyoğlu | 37º17'427" | 36º47'333" | 505 | *M. incognita* (Est: I1) | Race 2 |
| *103D* | Tomato | Ekinözü/Centre | 38º02'831" | 11º33'7" | 1251 | *M. incognita* (Est: I2) | Race 1 |

*Est: Esterase phenotypes

plant, *M. incognita* race 1 and race 2 in pepper plant were determined. Race determination for the populations obtained from Kahramanmaraş was made for the first time in located in the Eastern Mediterranean Region. In previous studies of race detection in our country, *M. incognita* race 1, race 2,

race 4, race 5, race 6 and *M. javanica* race 1 and race 5 were reported (Söğüt & Elekçioğlu, 2000; Mennan & Ecevit, 2001; Devran & Söğüt, 2011; Kaçar, 2011). In the study of Çetintaş & Çakmak, (2016), only the species were identified in Türkoğlu/Aydın kavak and Dulkadiroğlu/Çiğli, while in this study, the race of *M. incognita* was determined according to the North Carolina Differential Host Test (race 1). Gurkan et al. (2019) detected race 1, race 2, race 3 of *M. incognita*, race 3 of *M. javanica* and race 1 and race 3 of *M. arenaria* from 20 populations examined in the Mediterranean regio.

This study showed that precise diagnostic of root-knot nematode relies not only on morphological features but also on other techniques including molecular and biochemical methods. Identification studies are mostly time-consuming and need much professional skills (Blok et al. 2002). Furthermore, perineal patterns of some species are closely similar to each other which makes the morphological identifications incomplete. Nevertheless, it is needed for the confirmation of the other identification steps. Thus, controlling the root-knot nematodes requires rapid and precise analysis tools. In this study, species of *Meloidogyne incognita* and *M. javanica* that are very important parasites of vegetables and lead to high crop losses in Kahramanmaraş province were determined by applying SCAR primers.

*Meloidogyne* spp. is one of the key plant parasitic nematode groups becoming a growing concern for vegetable producers. Control of parasitic nematodes depends on detection ability and accurate diagnosis of nematode species to apply suitable and sustainable management methods. This is the first report of *M. javanica* on tomato and eggplant in Kahramanmaraş province. These findings show a potential risk of nematode presence and possible crop losses in eggplant and tomato growing areas in this region. Setting up cultural control techniques will be needed to reduce infestation around these areas. It is suggested that more effective control management tactics such as the use of resistant varieties and crop rotation should be applied. In addition, an integrated nematode management approach involving the combination of two or more suitable approaches using locally available resources in an integrated form can be necessary to deal with the threat of *Meloidogyne* spp. in vegetable areas.

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## Author's Contributions

The contribution of the authors is equal.

## Statement of Conflict of Interest

The authors declare no conflict of interest.

## REFERENCES

Adam, M.A.M., Phillips, M.S., & Blok, V.C. (2007). Molecular diagnostic key for identification for single juveniles of seven common and economically important species of root-knot nematode (*Meloidogyne* spp.), *Plant Pathology*. *56*, 190-197. https://doi.org/10.1111/j.1365-3059.2006.01455.x.

Adzitey, F., Huda, N., & Ali, G.R.R. (2013). Molecular techniques for detecting and typing of bacteria, advantages, and application to foodborne pathogens isolated from ducks. *3 Biotech*, *3*(2), 97-107. DOI: 10.1007/s13205-012-0074-4.

Aslan, A., & Elekçioğlu, İ.H. (2022). Biochemical and molecular identification of root-knot nematodes in green house vegetable areas of Eastern Mediterranean Region (Turkey). *Turkish Journal of Entomology*. *46*(1), 115-127. https://doi.org/ 10.16970/entoted.1055181.

Blok, V.C., Wishart, J., Fargette, M., Berthier, K., & Philips, M.S. (2002). "Mitochondrial DNA differences distinguishing *Meloidogyne mayaguensis* from the major species of tropical root-knot nematodes." *Nematology*. *4*(7), 773-781. https://doi.org/ 10.1163/156854102760402559.

Carneiro, R.M.D.G., & Almeida, M.R.A. (2001). Técnica de eletroforese usada no estudo de enzimas dos nematoides das galhas paraidentificação de especies. *Nematologia Brasileira. 25*, 35–44.

Carneiro, R.M.D.G., & Cofcewicz, E.T. (2008). Taxonomy of coffee-parasitic root-knot nematodes, *Meloidogyne* spp. In Plant Parasitic Nematodes of Coffee; Souza, R.M., Ed.; Springer: New York, NY, USA. 87–122. https://doi.org/10.1007/978-1-4020-8720-2_6.

Çetintas, R., & Çakmak, B. (2016). *Meloidogyne* species infesting tomatoes, cucumbers and eggplants grown in Kahramanmaraş Province, Turkey. *Türkiye Entomoloji Dergisi*. *40*(4), 355-364. DOI: http://dx.doi.org/10.16970/ted.40839.

Collange, B., Navarrete, M., Peyre, G., Mateille, T., & Tchamitchian, M. (2011). Root-knot nematode (*Meloidogyne*) management in vegetable crop production: The challenge of an agronomic system analysis. *Crop Protection*. *30*, 1251-1262. https:// doi.org/ 10.1016/j.cropro.2011.04.016.

Correa, V.R., Santos, M.F.A., Almeida, M.R.A., Peixoto, J.R., Castagnone-Sereno, P., & Carneiro, R.M.D.G. (2013). Species-specific DNA markers for identification of two root-knot nematodes of coffee: *Meloidogyne arabicida* and *M. izalcoensis*. *European Journal Plant Pathology*. *137*, 305–313.

DOI: 10.1007/s10658-013-0242-3.

Correa, V.R., Mattos, V.S., Almeida, M.R.A., Santos, M.F.A., Tigano, M.S., Castagnone-Sereno, P., & Carneiro, R.M.D.G. (2014). Genetic diversity of the root-knot nematode *Meloidogyne ethiopica* and development of a species-specific SCAR marker for its diagnosis. *Journal Plant Pathology*. *63*, 476–483. https://doi.org/10.1111/ppa.12108.

Devran, Z., & Söğüt, M.A. (2011). Characterizing races of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* in the West Mediterranean region of Turkey. *Crop Protection*. *30*(4), 451-455. https://doi.org/10.1016/j.cropro.2010.12.008.

Eisenback, J.D., & Triantaphyllou, H. (1991). Root-knot nematode: *Meloidogyne* sp. and races. In: Nickle WR, editor. *Manual of Agricultural Nematology*. New York: Marcel Decker; 191–274. DOI:10.1201/9781003066576-6.

Esbenshade, P.R., & Triantaphyllou, A.C. (1985). Use of enzyme phenotypes for identification of *Meloidogyne* species (Nematoda: Tylenchida), *Journal of Nematology*. *17*(1), 6-20.

Esbenshade, P.R., & Triantaphyllou, A.C. (1990). Isozyme phenotypes for the identification of *Meloidogyne* species. *Journal of Nematology*. *22*(1), 10-15.

Gerič Stare, B., Strajnar, P., Širca, S., Susič, N., & Urek, G. (2018). Record of a new location for tropical root knot nematode *Meloidogyne luci* in Slovenia. *EPPO Bulletin*. *48*(1), 135-137. https://doi.org/10.1111/epp.12443.

Gerič Stare, B., Aydınlı, G., Devran, Z., Mennan, S., Strajnar, P., Urek, G., & Širca, S. (2019). Recognition of species belonging to *Meloidogyne ethiopica* group and development of a diagnostic method for its detection. *European Journal of Plant Pathology*. *154*(3), 621-633. DOI: 10.1007/s10658-019-01686-2.

Gowda, M.T., Sellaperumal, C., Rai, A.B., & Singh, B. (2019). Root knot nematodes menace in vegetable crops and their management in India: A Review. *Vegetable Science*. *46*(1-2), 1-16.

Gürkan, B., Çetintaş, R., & Gürkan, T. (2019). Gaziantep ve Osmaniye Sebze Alanlarında Bulunan Kök-ur Nematodu Türleri (*Meloidogyne* spp.)'nin Teşhisi ile Bazı Nematod Popülasyon Irklarının Belirlenmesi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*. *22*(Ek Sayı 1), 113-124. https://doi.org/10.18016/ksutarimdoga.v22i49073.551240.

Hartman, K.M., & Sasser, J.N. (1985). Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology, '69-79'. An Advanced Treatise on *Meloidogyne*, vol II, Methodology, Eds.: K.R. Barker, C.C. Carter and J.N. Sasser. North Carolina State University Graphics. 223.

Hooper, D.J. (1986). Extraction of free-living stages from soil. In J.F. Southey, Ed. Laboratory Methods for Work with Plant and Soil Nematodes. Ministry of Agriculture Fisheries and Food, Reference Book. 402.

Hunt, D.J., & Handoo, Z.A. (2009). "Taxonomy, Identification and Principal Species, 55-97". Root-knot Nematodes, (Eds. R. N. Perry, M. Moens and J. L. Starr). 1st Ed. Wallingford, UK: CAB International. 97. https://doi.org/10.1079/9781845934927.0055.

Kaçar, G. (2011). *Türkiye'de Bulunan Kök-ur Nematodu Türlerinin (Meloidogyne spp.) (Nemata: Meloidogynidae) Irklarının Araştırılması.* Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Bitki Koruma Ana Bilim Dalı, Yüksek Lisans Tezi, 49.

Mennan, S., & Ecevit, O. (2001). Bafra ve Çarşamba Ovaları'ndan elde edilen bazı *Meloidogyne incognita* (Kofoid and White, 1919) (Nemata: Heteroderidae) popülasyonlarında ırk tespiti. *Türkiye Entomoloji Dergisi*. *25*(1), 33-39. https://hdl.handle.net/20.500.12712/8389.

Özarslandan, A., & Elekçioğlu, İ.H. (2010). Türkiye'nin farklı alanlarından alınan kök-ur nematodu türlerinin (*Meloidogyne* spp.) (Nemata: Meloidogynidae) moleküler ve morfolojik tanılama ile belirlenmesi, *Türkiye Entomoloji Dergisi*. *34*(3), 323-335.

Powers, T.D., & Harris, T.S. (1993). A polymerase chain reaction method for identification of five major *Meloidogyne* species. *Journal of Nematology*. *25*, 1-6.

Powers, T.O., Todd, T.C., Burnell, A.M., Murray, P.C., Fleming, C.C., Szalanski, A.L., & Harris, T.S. (1997). The rDNA Internal Transcribed Spacer Region as a Taxonomic Marker for Nematodes. *Journal of Nematology*. *29*(4), 441-450.

Randig, O., Bongiovanni, M., Carneiro, R.M.D.G., & Castagnone-Sereno, P. (2002). Genetic diversity of root-knot nematodes from Brazil and development of SCAR markers specific for the coffee-damaging species, Genome. *45*, 862-70. DOI: 10.1139/g02-054.

Rao, M.S., Umamaheswari, R., Priti, K., Rajinikanth, R., Vidyashree, P., Prabhu Kamalnath, M. (2015). Nematode management in vegetable crops. *IIHR Technical Bulletin*. Published by Director ICAR-IIHR, Bengaluru. No:47.

Resquin-Romero, G., Mattos, V.S., Monteiro, J.M.S., Lopez-Nicora, H.D., Amarilla, S.P., Chamorro-Diaz, S., Moral, J., & Carneiro, R.M.D.G. (2023). Enzymatic and Molecular Identification of *Meloidogyne* Species in Tomato Orchards in Paraguay. *Agronomy*. *13*, 670. https://doi.org/10.3390/agronomy13030670.

Roberts, P.A. (1992). Current status of the availability, development, and use of host plant resistance to nematodes, *Journal of Nematology*. *24*(2), 213–27.

Salgado, S.M.L., Guimaraes, N.M.R.B., Botelho, C.E., Tassone, G.A.T., Marcelo, A.L., Souza, S.R., Oliveira, R.D.L., & Ferreira, D.F. (2015). *Meloidogyne paranaensis e Meloidogyne exigua* em lavouras cafeeiras da região Sul de Minas Gerais. *Coffee Science*. *10*, 475-481.

Shaaban, M.H., Mahdy, M.E.S., Selim, M.E., & Mousa, El-S.M. (2023). Pathological, Chemical and Molecular Analysis of Eggplant Varieties Infected with Root-knot Nematodes (*Meloidogyne* spp.). *Egyptian Journal of Crop Protection*. *18*(1), 1-13. DOI: 10.21608/EJCP.2023.174909.1013.

Sijmons, P.C., Atkinson, H.J., & Wyss, U. (1994). Parasitic strategies of root–knot nematodes and associated host cell responses, *Annual Review of Phytopathol*ogy. *32*, 235-59. DOI: 10.1146/ annurev.py.32.090194.001315.

Söğüt, M., & Elekçioğlu, I.H. (2000). Determination of *Meloidogyne* Goeldi, 1892 (Nemata: Heteroderidae) species races found in vegetable growing areas of the Mediterranean region of Turkey. *Turkish Journal of Entomology*. *24,* 33-40.

Tapia-Vázquez, I., Montoya-Martínez, A.C., De los Santos-Villalobos, S., Ek-Ramos, M.J., Montesinos-Matías, R., & Martínez-Anaya, C. (2022). Root-knot nematodes (*Meloidogyne* spp.) a threat to agriculture in Mexico: Biology, current control strategies, and perspectives. *Journal Microbiology*. Biotechnology. *38*, 26. DOI: 10.1007/s11274-021-03211-2.

Thorne, G. (1961). Principles of Nematology, New York. 312-21.

Tigano, M., Siqueira, K., Castagnone-Sereno, P., Mulet, K., Queiroz, P., Santos, M., Teixeira, C., Almeida, M., Silva, J., & Carneiro, R.M.D.G. (2010). Genetic diversity of the root-knot nematode *Meloidogyne enterolobii* and development of a SCAR marker for this guava-damaging species. *Plant Pathology*. *59*, 1054–1061. DOI: 10.1111/j.1365-3059.2010.02350x.

Tytgat, T., Meutter, J.D., Gheysen, G., & Coomans, A. (2000). Sedentary endoparasitic nematodes as a model for other plant parasitic nematodes, *Nematology*. *2*(1), 113–21. DOI: 10.1163/ 156854100508827.

Tzortzakakis, E.A., Blok, V.C., Phillips, M.S., & Trudgill, D.L. (1999). Variation in Root-Knot nematode (*Meloidogyne* spp.) in Crete in relation to control with resistant tomato and pepper, *Nematology*. *1*(5), 499–506.

Wang, Y., Yang, W., Zhang, W., Han, Q., Feng, M., & Shen, H. (2013). Mapping of a heat-stable gene for resistance to southern root-knot nematode in *Solanum lycopersicum*. *Plant Molecular Biology Reporter*. *31*(2), 352-362. DOI: 10.1007/s11105-012-0505-8.

Young, L.D. (1992). Problems and strategies associated with long-term use of nematode resistant cultivars, *Journal of Nematology*. *24*(2), 228-33.

Zijlstra, C., Lever, A.E.M., Uenk, B.C., & Van Silfhout, C.H. (1995). Differences between ITS regions of isolates of root-knot nematodes *Meloidogyne hapla* and *M. chitwoodi*. *Phytopathology*. *85*:1231-1237. DOI:10.1094/PHYTO-85-1231.

Zijlstra, C., Donkers-Venne, D.T.H.M., & Fargette, M. (2000). Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays, *Nematology*. *2*, 847-53. DOI: 10.1163/ 156854100750112798