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Distribution and Identification of Root-knot Nematodes from Turkey

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Abstract: Root knot nematodes are causing serious losses in protected cultivation fields in the West Mediterranean region of Turkey. Correct and confident identification of the plant parasitic nematodes is important for vegetable growing and breeding. Therefore, ninety-five populations of plant parasitic nematodes were collected from regional greenhouses. Previously described species-specific primers were used to identify *Meloidogyne* populations. The present study indicated that SEC-1F/SEC-1R and INCK14F-INCK14R primers for identifying of *M. incognita*, Fjav/Rjav and DJF/DJR primers for *M. javanica* and Far/Rar for *M. arenaria* primers can be effective tools to identify the Turkish root-knot nematode species. Dissemination ratios of the population were 64.2%, 28.4% and 7.3% for *Meloidogyne incognita*, *M. javanica* and *M. arenaria*, respectively. The results showed that *M. incognita* was the prominent root-knot nematode species in the West Mediterranean coastal areas of Turkey.

Key words: Distribution, diagnosis, PCR technique, root-knot nematode, Turkey.

Turkey is amongst the leading vegetable producers in the world. Total vegetable production was nearly 22 million metric tones produced from 815.000 ha (Anonymous, 2007). Vegetables are produced both in open fields and protected fields in Turkey. Turkey is geographically divided into seven regions including the Mediterranean that is further divided into two regions which includes the West Mediterranean and East Mediterranean regions. The West Mediterranean region is the centre of vegetable production due to its climatic conditions.

The root-knot nematode group is one of the most important pathogen affecting crops in protected cultivation in the Mediterranean coastal areas of Turkey (Elekçioğlu and Uygun 1994; Elekçioğlu et al., 1994; Devran et al., 2008). Over the past decade, a number of studies have been conducted on root-knot nematodes in Turkey (Elekçioğlu and Uygun, 1994; Elekçioğlu et al., 1994; Mennan and Ecevit, 1996; Söğüt and Elekçioğlu, 2000; Devran et al., 2002; Devran et al., 2008). However, a comprehensive work, so far, has not been done on the distribution and diagnosis of the nematode in the West Mediterranean region of Turkey.

Correct identification of plant parasitic nematodes is of importance in terms of vegetable growing and breeding. Root-knot nematodes have been identified based on morphological characters (Eisenback and Triantaphyllou, 1991), host-plant response (Hartman and Sasser, 1985), isozyme analyses (Esbenshade and Triantaphyllou, 1990) and molecular techniques (Powers and Harris, 1993; Powers et al., 1997; Ziljstra et al., 1995; Zijlstra et al, 2000; Adam et al., 2007). However, identification by morphological characters and host plant response is time consuming and needs extensive labor. Isozymes analysis is an effective method which can be carried out on females of *Meloidogyne* spp. (Esbenshade and Triantaphyllou, 1990). However, application of this method only on female individuals is a limited factor, is difficult, and can be affected by environmental factors. However, molecular techniques based on DNA can be used in every stage of the nematode's life cycle, and they are rapid, and reliable.

The objectives of this study were to identify root-knot nematodes collected from different locations of the West Mediterranean coastal areas of Turkey using molecular methods, and to select effective primers from previous studies for *Meloidogyne* species identification in this region. Furthermore, regional distribution of the nematode was determined.

Materials and Methods

Root-knot nematode cultures: Ninety-five populations of *Meloidogyne* spp. from the Mediterranean costal areas of Turkey were collected from the roots of infested cultivated host plants (Table 1, Figure 1). Egg masses were picked up from roots using a small needle. Each root-knot nematode isolate was established as single mass line in monoxenic pure cultures. Root-knot nematodes were reared on fresh market tomato plants (Tueza F1, Multi Tarım, Turkey). Tomato seeds were germinated in steam-sterilized sandy soil in seed tray, and 2-wk-old seedlings were transplanted singly into 250 ml plastic pots. Plants were inoculated at the fourth true leaf stage with single nematode egg mass.

DNA extraction: DNA was extracted from nematode egg masses, juveniles and females according to DNAeasy Tissue and Blood Kit (Qiagen, Hilden, Germany) following manufacturer' instructions.

Species-Specific PCR: The primers used for DNA analysis of the root-knot nematode species are listed in Table 2. All PCR amplification was performed in a total volume of 25 µl containing 10XPCR Buffer, 0.2 mM dNTP, 0,4 µM of each primer, 2 mM $MgCI_2$, 20 ng of template DNA and 1 Unit Taq DNA Polymerase (Vivantis). The PCR amplification condition of primers are described in Table 3. PCR was carried out using a thermal cycler DNA Engine PTC-200 Peltier Thermal Cycler (Bio-Rad, Hercules, CA). PCR products were separated

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TABLE 1. Root-knot nematodes populations used in this study

Population Code	Location	Host Plant	Species	Population Code	Location	Host Plant	Species
G2	Gazipaşa	Tomato	*M. incognita*	KA7	Kaş	Tomato^R	*M. incognita*
G3	Gazipaşa	Cucumber	*M. incognita*	F1	Fethiye	Tomato	*M. incognita*
G4	Gazipaşa	Eggplant	*M. incognita*	F2	Fethiye	Tomato	*M. incognita*
G7	Gazipaşa	Tomato	*M. incognita*	F6	Fethiye	Tomato	*M. incognita*
A2	Alanya	Tomato	*M. incognita*	F9	Fethiye	Tomato	*M. incognita*
A3	Alanya	Cucumber	*M. incognita*	F10	Fethiye	Tomato	*M. incognita*
A6	Alanya	Cucumber	*M. incognita*	F11	Fethiye	Tomato	*M. incognita*
A8	Alanya	Cucumber	*M. incognita*	F12	Fethiye	Tomato	*M. incognita*
S1	Serik	Tomato	*M. incognita*	F13	Fethiye	Tomato	*M. incognita*
S2	Serik	Tomato	*M. incognita*	F15	Fethiye	Tomato	*M. incognita*
S3	Serik	Tomato	*M. incognita*	O2	Ortaca	Tomato	*M. incognita*
S4	Serik	Tomato	*M. incognita*	O5	Ortaca	Tomato	*M. incognita*
S5	Serik	Tomato	*M. incognita*	G1	Gazipaşa	Tomato	*M. javanica*
S6	Serik	Pepper	*M. incognita*	G5	Gazipaşa	Eggplant	*M. javanica*
M1	Gaziler	Tomato	*M. incognita*	A1	Alanya	Eggplant^R	*M. javanica*
M2	Gaziler	Tomato	*M. incognita*	A4	Alanya	Cucumber	*M. javanica*
M3	Gaziler	Tomato	*M. incognita*	A5	Alanya	Tomato	*M. javanica*
M4	Gaziler	Tomato	*M. incognita*	A7	Alanya	Cucumber	*M. javanica*
M5	Gaziler	Tomato	*M. incognita*	AKS1	Aksu	Tomato	*M. javanica*
M6	Gaziler	Tomato	*M. incognita*	AKS2	Aksu	Tomato	*M. javanica*
M10	Altınova	Tomato	*M. incognita*	AKS3	Aksu	Tomato^R	*M. javanica*
K1	Kumluca	Eggplant	*M. incognita*	AKS4	Aksu	Tomato	*M. javanica*
K2	Kumluca	Pepper	*M. incognita*	AKS5	Aksu	Tomato	*M. javanica*
K3	Kumluca	Pepper	*M. incognita*	AKS6	Aksu	Tomato	*M. javanica*
K4	Kumluca	Pepper	*M. incognita*	AKS7	Aksu	Tomato	*M. javanica*
K5	Kumluca	Pepper	*M. incognita*	M7	Altınova	Tomato	*M. javanica*
K6	Kumluca	Pepper	*M. incognita*	M8	Altınova	Tomato	*M. javanica*
K7	Kumluca	Pepper	*M. incognita*	M11	Altınova	Tomato	*M. javanica*
K8	Kumluca	Pepper	*M. incognita*	K13	Finike	Tomato	*M. javanica*
K9	Kumluca	Pepper	*M. incognita*	K16	Kumluca	Tomato	*M. javanica*
K10	Kumluca	Tomato^R	*M. incognita*	K20	Finike	Cucumber	*M. javanica*
K11	Kumluca	Pepper	*M. incognita*	K21	Finike	Cucumber	*M. javanica*
K12	Kumluca	Tomato	*M. incognita*	D3	Demre	Tomato	*M. javanica*
K14	Kumluca	Pepper	*M. incognita*	KA6	Kaş	Tomato^R	*M. javanica*
K15	Kumluca	Eggplant	*M. incognita*	F4	Fethiye	Tomato	*M. javanica*
K17	Kumluca	Tomato	*M. incognita*	F5	Fethiye	Tomato	*M. javanica*
K19	Kumluca	Tomato	*M. incognita*	F7	Fethiye	Tomato	*M. javanica*
D1	Demre	Tomato	*M. incognita*	F8	Fethiye	Tomato	*M. javanica*
D2	Demre	Tomato	*M. incognita*	F14	Fethiye	Tomato	*M. javanica*
D4	Demre	Tomato	*M. incognita*	O3	Ortaca	Tomato	*M. javanica*
D5	Demre	Tomato	*M. incognita*	M9	Altınova	Tomato	*M. arenaria*
D6	Demre	Bean	*M. incognita*	K18	Kumluca	Tomato	*M. arenaria*
D7	Demre	Bean	*M. incognita*	K22	Finike	Cucumber	*M. arenaria*
KA1	Kaş	Tomato	*M. incognita*	O1	Ortaca	Tomato	*M. arenaria*
KA2	Kaş	Pepper	*M. incognita*	O4	Ortaca	Tomato	*M. arenaria*
KA3	Kaş	Pepper	*M. incognita*	O6	Ortaca	Tomato	*M. arenaria*
KA4	Kaş	Pepper	*M. incognita*	O7	Ortaca	Tomato	*M. arenaria*
KA5	Kaş	Tomato^R	*M. incognita*				

Tomato^R: Tomato rootstock, Eggplant^R: Eggplant rootstock

by electrophoresis in Tris-EDTA (TAE) buffer with 2 % agarose gel stained with ethidium bromide (0.5 μg/ml) at 100 V for 2.5 h and then visualized under UV light.

RESULTS

Molecular Characterization: The primer pairs used for diagnosis of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* were screened for species specificity. The optimized primers resulted in consistent amplifications by DNA obtained from egg masses, juveniles and females. In this study, gene specific and SCAR primers were used for identification of root-knot nematodes. Tesarova et al. (2003) reported that Sec primer was developed from SEC protein gene sequences belonging to *M. incognita*. C2F3 and 1108 primers were developed from mitochondrial gene sequence (Powers and Harris, 1993). Other primers were species-specific SCAR developed from RAPD markers (Zijlstra et al., 2000; Dong et al., 2001; Randig et al., 2002; Meng et al., 2004).

Although some primer sets resulted in positive amplifications, there were also some primers giving no product, therefore these primer sets were not used for root-knot nematode identification.

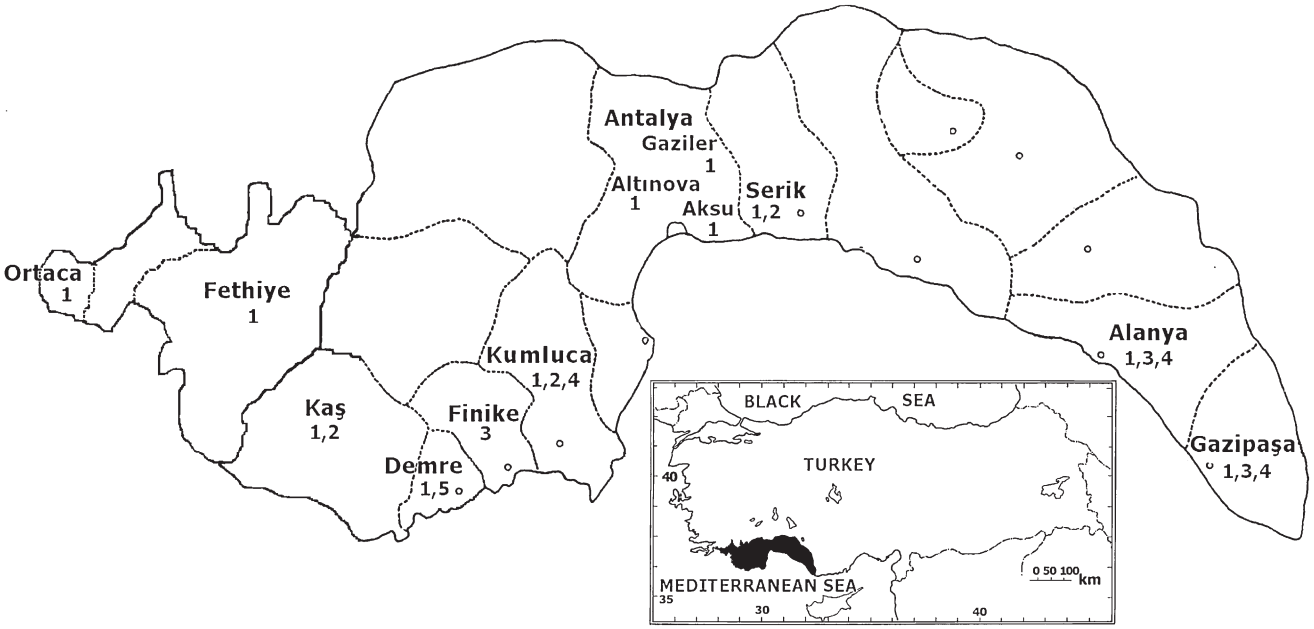

FIG. 1. The West Mediterranean Region map. 1: Tomato, 2: Pepper, 3: Cucumber, 4: Eggplant and 5: Bean.

Meloidogyne incognita was identified using five different primer sets (Table 2). PCR with the *M. incognita* species-specific SEC-1F/SEC-1R, inc-K14-F/ inc-K14-R and MIF/MIR produced approximately 500 bp, 400 bp and 1000 bp DNA fragment for all *M. incognita* populations, respectively (Figs. 2-4). However, Finc/Rinc and DIF/DIR primer sets did not display amplification. Whilst *M. javanica* was identified by Fjav-Rjav and DJF/DJR primer sets produced 670 bp and 1650 bp fragment from all populations, respectively (Fig. 5A, B), there were no amplification products with C2F3-1108 primers. The Far/Rar primer set produced a 420 bp fragment, which was characteristic in all populations of *M. arenaria*. However, DAF/DAR and C2F3/1108 primers did not give amplification products in *M. arenaria* populations (Fig. 6).

Distribution of the pathogen: Meloidogyne incognita, *M. javanica* and *M. arenaria* ratios were 64.2%, 28.4% and 7.3% of the total populations, respectively. *Meloidogyne incognita* was determined as the most common root-knot

TABLE 2. Primers used for molecular identification of root-knot nematode

Name of Primer	Species	Fragment (bp)	Primer Sequences (5-3)	References
Finc/Rinc	*M. incognita*	1200	CTCTGCCCAATGAGCTGTCC CTCTGCCCTCACATTAGG	Zijlstra et al., (2000)
DIF/DIR*	*M. incognita*	1350 and 1370	TAGGCAGTAGGTTGTCGGG CAGATATCTCTGCATTGGTGC	Dong et al., 2001
MIF/MIR	*M. incognita*	999	GTGAGGATTCAGCTCCCCAG ACGAGGAACATACTTCTCCGTCC	Meng et al., 2004
inc-K14F inc-K14R	*M. incognita*	399	CCCGCTACACCCTCAACTTC GGGATGTGTAAATGCTCCTG	Randig et al., 2002
SEC-1F SEC-1R	*M. incognita*	502	GGGCAAGTAAGGATGCTCTG GCACCTCTTTCATAGCCACG	Teserova et al., 2003
Fjav/Rjav	*M. javanica*	670	GGTGCGCGATTGAACTGAGC CAGGCCCTTCAGTGGAACTATAC	Zijlstra et al., 2000
DJF/DJR*	*M. javanica*	1650	CCTTAATGTCAACACTAGAGCC GGCCTTAACCGACAATTAGA	Dong et al., 2001
C2F3/1108	*M. javanica* *M. arenaria*	1500 1100	GGTCAATGTTCAGAAATTTGTGG TACCTTTGACCAATCACGCT	Power and Harris, 1993
Far/Rar	*M. arenaria*	420	TCGGCGATAGAGGTAAATGAC TCGGCGATAGACACTACAACT	Zijlstra et al., 2000
DAF/DAR*	*M. arenaria*	950	TCGAGGGCATCTAATAAAGG GGGCTGAATATTCAAAGGAA	Dong et al., 2001

*Primers developed by Dong et al., (2001) has been indicated as DIF/DIR, DJF/DJR and DAF/DAR in the present table by expecting their intelligence and permissions, since any specific acronym has not been given in their published article.

TABLE 3. PCR amplification conditions of primers used for molecular identification of root-knot nematode

Name of Primer	Amplification Conditions	
inc-K14-F/inc-K14-R	94 °C 3 min	
Fjav/Rjav	94 °C 30 sec	
MIF/MIR	60 °C 30 sec	35 cycles
	72 °C 60 sec	
	72 °C 7 min	
SEC-1F/SEC-1R	94 °C 3 min	
Far/Rar	94 °C 30 sec	
	56 °C 30 sec	35 cycles
	72 °C 60 sec	
	72 °C 7 min	
DJF/DJR	94 °C 3 min	
	94 °C 30 sec	
	52 °C 30 sec	35 cycles
	72 °C 120 sec	
	72 °C 7 min	

nematode species in the Mediterranean coastal areas of Turkey. *Meloidogyne incognita* was also detected in all districts in which tomato, pepper, cucumber and eggplant cultivars are grown. *Meloidogyne javanica* was detected in every district except for Serik. *Meloidogyne javanica* was not detected within samples collected from pepper growing areas. *Meloidogyne arenaria* was especially found in Ortaca where the tomato is cultivated.

DISCUSSION

Root-knot nematode species are morphologically very similar to each other and identification to the species level is difficult. Moreover, more than one root knot nematode species are sometimes found together in the same plant root. Therefore, fast and accurate identification of root-knot nematodes is needed for management and breeding. Root-knot nematode species, *M. incognita*, *M. javanica* and *M. arenaria*, *which* cause serious yield losses in protected vegetable areas in

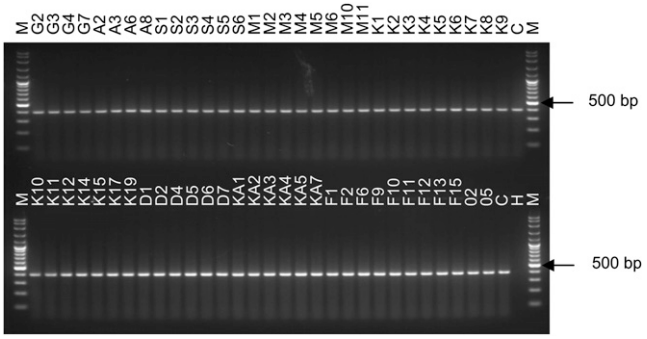

FIG. 3. Amplification products with the *M. incognita* species-specific inc-K14-F/ inc-K14-R primers. M: 100 bp DNA Ladder (Vivantis), Samples (G2-O5), C: Positive control, H: water

Mediterranean coastal areas of Turkey, were identified using species-specific primers in this study. Primers optimized successfully resulted in amplification of DNA obtained from all the nematode sources including egg masses, juveniles and females.

Different primer sets were used in our studies to identify of root-knot nematodes. SEC-1F/SEC-1R, inc-K14-F/inc-K14-R, MIF/MIR for *M. incognita*, Fjav/Rjav, DJF/DJR and Far/Rar primers for *M. arenaria* gave successfully PCR products. Our results showed agreement with earlier studies (Zijlstra et al., 2000; Dong et al., 2001; Teserova et al., 2003; Randig et al., 2002; Meng et al., 2004; Adam et al, 2005; Tzortzakakis et al., 2005; Adam et al., 2007). *Meloidogyne incognita* species-specific MIF/MIR primer effectively gave the expected DNA fragment in all *M. incognita* populations. Moreover, this primer successfully gave PCR products in some *M. javanica* populations (A1, A4, A5, AKS2, AKS7). This primer was reported as a specific primer for *M. incognita* (Meng et al., 2004, Adam et al., 2007). This study showed that this primer was not specific for identification of *M. incognita* which might be due to differences in the binding sites of the primers.

Although the primers Finc/Rinc (Zijlstra et al., 2000, Adam et al, 2005), DIF/DIR (Dong et al., 2001), C2F3/

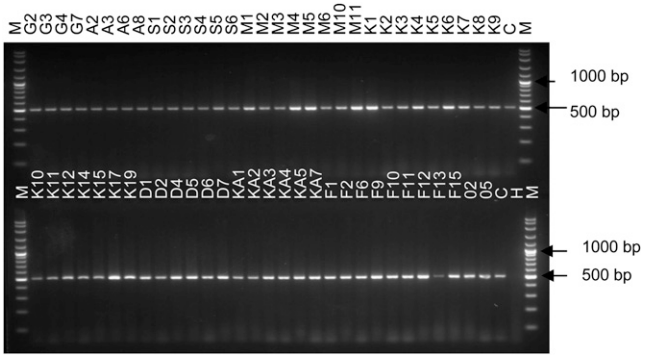

FIG. 2. Amplification products with the *M. incognita* species-specific SEC-1F/SEC-1R primers. M: 100 bp DNA Ladder (Vivantis), Samples (G2-O5), C: Positive control, H: water

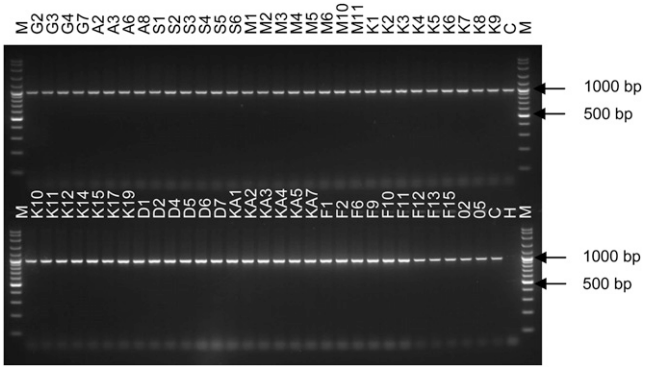

FIG. 4. Amplification products with the *M. incognita* species-specific MIF-MIR primers. M: 100 bp DNA Ladder (Vivantis), Samples (G2-O5), C: Positive control, H: water

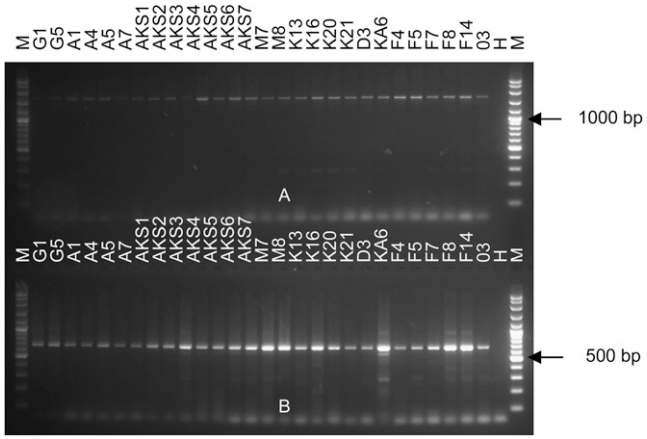

Fig. 5A. PCR products with the *M. javanica* species-specific DJF-DJR primers. M: 100 bp DNA Ladder (Vivantis), Samples (G1-O3), H: water

Fig. 5B. PCR products with the *M. javanica* species-specific Fjav-Rjav primers M: 100 bp DNA Ladder (Vivantis), Samples (G1-O3), H: water

1108 (Powers and Harris, 1993, Powers et al., 2005) and DAF/DAR (Dong et al., 2001) were previously tested and reported as effective primers, no amplification product was obtained by these primers in our study. Amplification could have failed due to changes in the priming sites and PCR conditions. Obtaining inconsistent product by Finc/Rinc primers has previously been reported (Adam et al., 2007) and our findings are in agreement with Adam et al. (2007).

In previous research, the authors reported that *M. incognita* was a common species in vegetables growing areas of Turkey (Elekçioğlu and Uygun, 1994; Elekçioğlu et al., 1994; Söğüt and Elekçioğlu, 2000, Mennan and Ecevit, 1996). Söğüt and Elekçioğlu, (2000) informed that *M. areneria* was rarely detected in protected cultivars. *Meloidogyne javanica* was also not founf in pepper cultivations of the East Mediterranean region (Söğüt and Elekçioğlu, 2000). Our study showed accordance with these previous findings.

In conclusion, our study reported on the optimization of PCR for routine, rapid and accurate identification of Turkish root-knot nematode species.

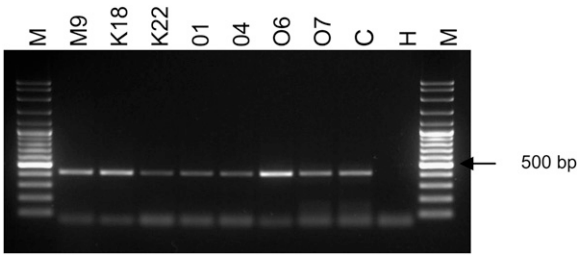

Fig. 6. Amplification products with the *M. arenaria* species-specific Far-Rar primers. M: 100 bp DNA Ladder (Vivantis), Samples (M9-O7), C: Positive control, H: water

Literature Cited

Adam, M. A. M., Phillips, M. S., and Blok, V. C. 2007. Molecular diagnostic key for identification of single juveniles of seven common and economically important species of root-knot nematode (*Meloidogyne* spp). Plant Pathology 56:190–197.

Anonymous. 2007. Crop production statistics. Turkish Statistical Institute, Ankara, Turkey. http://www.tuik.gov.tr/VeriBilgi.do?tb_id=45&ust_id=13.

Devran, Z., Gözel, U., Söğüt, M. A., Yıldız, Ş., and Elekçioğlu, İ.H. 2002. Identification of root-knot nematodes in the Mediterranean Region of Turkey by using rDNA and mtDNA markers. Turkish Journal of Agriculture and Forestry 26:337–341.

Devran, Z., Söğüt, M. A., Gözel, U., Tör, M., and Elekçioğlu, İ.H. 2008. Analysis of genetic variation between populations of *Meloidogyne* spp. from Turkey. Russian Journal of Nematology 16:143–149.

Dong, K., Dean, R. A., Fortnum, B. A., and Lewis, S. A. 2001. Development of PCR primers to identify species of root-knot nematodes: *Meloidogyne arenaria, M. hapla, M. incognita*, and *M. javanica*. Nematropica 31:271–280.

Esbenshade, P. R., and Triantaphyllou, A. C. 1990. Isozyme phenotypes for the identification of *Meloidogyne* species. Journal of Nematology 22:10–15.

Eisenback, D. E., and Triantaphyllou, H. H. 1991. Root-knot nematodes: Meloidogyne species and races. Pp. 191–274. *in* W. R. Nickle, ed. Manual of Agricultural Nematology, New York: Marcel Dekker Inc.

Elekçioğlu, İ. H., and Uygun, N. 1994. Occurrence and distribution of plant-parasitic nematodes in cash crop in Eastern Mediterranean region of Turkey. Proceeding of 9th Congress of The Mediterranean Phytopathological Union, Kuşadası -Turkey 409–410.

Elekçioğlu, İ. H., Ohnesorge, B., Lung, G., and Uygun, N. 1994. Plant parasitic nematodes in the Mediterranean region of Turkey. Nematologia Mediterranea 22:59–63.

Hartman, K. M., and Sasser, J. N. 1985. Pp. 69–77. *in* K. R. Barker, C. C. Carter, and J. N. Sasser, eds. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. An Advanced Treatise on Meloidogyne Volume II: *Methodology*. North Carolina State University Graphics.

Meng, Q. P., Long, H., and Xu, J. H. 2004. PCR assays for rapid and sensitive identification of three major root-knot nematodes, *Meloidogyne incognita, M. javanica* and *M. arenaria*. Acta Phytopathologica Sinica 34:204–210.

Mennan, S., and Ecevit, O. 1996. Studies on biology, distrubition and the ratio of infestation of root-knot nematodes (*Meloidogyne* spp.) in summer vegetable growing area in Bafra and Çarşamba plains. Proceedings of the Third Turkish National Congress of Entomology 700–705.

Powers, T. O., and Harris, T. S. 1993. A polymerase chain reaction method for dentification of five major *Meloidogyne* species. Journal of Nematology 25:1–6.

Powers, T. O., Todd, T. C., Burnell, A. M., Murray, P. C. B., Fleming, C. C., Szalanski, A. L., Adams, B. A., and Harris, T. S. 1997. The rDNA internal transcribed spacer region as a taxonomic marker for Nematodes. Journal of Nematology 29:441–450.

Powers, T. O., Mullin, P. G., Harris, T. S., Sutton, L. A., and Higgins, R. S. 2005. Incorporating molecular identification of *Meloidogyne* spp. into a large-scale regional nematode survey. Journal of Nematology 37:226–235.

Randig, O., Bongiovanni, M., Carneiro, R. M. D. G., and Castagnone-Sereno, P. 2002. Genetic diversity of root-knot nematodes from Brazil and development of SCAR marker specific for the coffee damaging species. Genome 45:862–870.

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

Tesarova, B., Zouhar, M., and Rysanek, P. 2003. Development of PCR for specific determination of root-knot nematode *Meloidogyne incognita*. Plant Protection Science 39:23–28.

Tzortzakakis, E. A., Adam, M. A. M., Blok, V. C., Paraskevopoulos, C., and Bourtzis, K. 2005. Occurrence of resistance-breaking populations of root-knot nematodes on tomato in Greece. European Journal of Plant Pathology 113:101–5.

Zijlstra, C., Lever, A. E. M., Uenk, B. J., and van Silfhout, C. H. 1995. Differences between ITS regions of isolates of root-knot nematodes *Meloidogyne chitwoodi* and *M. hapla*. Phytopathology 85:1231–1237.

Zijlstra, C., Donkers-Venne, D. T. H. M., and Fargette, M. 2000. Identification of *Meloidogyne incognita, M. javanica* and *M. arenaria* using sequence characterized amplified region (SCAR) based PCR assays. Nematology 2:847–853.