

FIRST REPORT OF ROOT-KNOT NEMATODES, *MELOIDOGYNE ARENARIA* AND *M. HAPLA* (NEMATA: MELOIDOGYNIDAE) FROM PEPINO IN TURKEY

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Summary. A survey was conducted during 2010-2011 to investigate the root-knot nematode species occurring in pepino (*Solanum muricatum* Aiton) (Solanaceae) growing areas in Ordu and Samsun provinces, in the Black Sea Region of Turkey. A moderate to severe root-knot infection was observed. Microscopic examination of galled roots revealed the presence of mature females. Extracted females were identified to the species level based on available perineal patterns, morphology, esterase (Est) phenotypes in PAGE (Polyacrylamide Gel Electrophoresis) and the identification confirmed molecularly. The root-knot nematode from Ordu area was identified as *Meloidogyne hapla* by the exact size of 440 bp amplicons by JMV hapla scar primer. The other *Meloidogyne* species from Samsun area was identified as *M. arenaria* based on the size, 420 bp, amplified by *M. arenaria* SCAR primer. *Meloidogyne arenaria* and *M. hapla* are reported for the first time on pepino in Turkey.

Key words: Identification, *Meloidogyne arenaria*, *M. hapla*, *Solanum muricatum*.

Meloidogyne spp. (root-knot nematodes) are economically important plant parasites affecting a broad range of host plants. Solanaceae plants (tomato, potato, eggplant, pepper, etc.) are considered among the main hosts for root-knot nematodes. The genus *Meloidogyne* is able to infect more than 5,500 plant species (Trudgill and Blok, 2001). *Meloidogyne arenaria* (Neal) Chitw. have been reported from tomatoes, cucumbers and eggplants in Turkey (Özarslandan and Elekçioğlu, 2010). *Meloidogyne hapla* has been reported in temperate climate areas of Central and Eastern Anatolia regions of Turkey (Yüksel, 1974; Elekçioğlu and Uygun, 1994; Elekçioğlu *et al.*, 1994; Sogut and Elekçioğlu, 2000). In Turkey, many hosts for *M. arenaria* and *M. hapla* have been recorded; however, infestation of pepino, *Solanum muricatum* Aiton (Solanaceae) by these nematode species has not yet been reported. In the world, however, *M. incognita* has been reported on pepino only in New Zealand (Knight *et al.*, 1997). There is an increasing production and commercialization in European fruit markets of this crop in recent years (Martinez-Romero *et al.*, 2003). Because of the increasing demand, several attempts have been made to introduce this crop into various regions having a Mediterranean climate (Prohens *et al.*, 1996). Pepino is very new and not yet grown adequately on a commercial scale in Turkey. In recent years, it has been grown in some small-scale greenhouses in Mediterranean and Black Sea regions of the country. In September of 2010-2011, typical root-knot nematode symptoms, such as stunting and extensive root galls, were found on the roots of pepino plants in Ordu

and Samsun provinces, Black Sea Region of Turkey. This was the first finding of root-knot nematode infection on pepino in Turkey. The objective of this study was to identify the *Meloidogyne* spp. found on pepino in Ordu and Samsun provinces of Turkey by morphological, biochemical and molecular methods.

MATERIAL AND METHODS

Nematode samples and preparation of perineal patterns. Plant samples were collected from two main pepino producing districts, Terme and Kayabaşı, of Samsun and Ordu provinces, respectively (Fig. 1). Root-knot nematode symptoms, such as stunting and extensive root galling were observed on roots of pepino plants (Fig. 2). *Meloidogyne* spp. females were dissected from the galls using a stereomicroscope (Leica, S8APO) at 40× magnification. Females were placed in a drop of 45% lactic acid and the perineal regions were cut and cleaned. Perineal patterns were prepared following the procedures suggested by Hartman and Sasser (1985). Morphological observations and photographs were completed within 24 hours following slide preparation with the help of a light microscope equipped with a digital camera (Leica, DM2500).

Electrophoresis, sample preparation, and loading. A Bio-Rad mini-PROTEAN II (Bio-Rad, Philadelphia, PA) electrophoresis unit was used. Before electrophoresis, the females were thawed and homogenized individually in micro-haematocrite plastic tubes in 10 ml of extraction buffer. Each sample was then loaded into a well. Each gel contained 10 wells. Standard *M. javanica* female extract was placed into wells 1 and 10. The remaining 8 wells

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Fig. 1. Map of the provinces where pepino plant samples were collected in Turkey.

were loaded with the protein extract from test sample females. Electrophoresis was conducted in a discontinuous buffer system with 8% acrylamide running gel, pH 8.8 and 4% acrylamide stacking gel, pH 6.8. The voltage was maintained at 80 volts for the first 16 minutes and increased to 200 volts for the remainder of the running period. Following electrophoresis, the gels were removed and placed in an enzyme reaction mixture to determine esterase patterns (Harris and Hopkinson, 1976). The malate dehydrogenase isozyme was also determined but, unfortunately, the data are not available.

DNA extraction and PCR-SCAR. Nematode DNA was extracted from each of the five female samples using the DNeasy Blood & Tissue kit (Qiagen, Maryland, USA). The final volume extracted from each sample was 30 μ l in EB buffer.

Species-specific amplicons were obtained using a total of 25 μ l of PCR reaction mixture with 2 units of *Taq* PCR Master Mix kit (Qiagen, Maryland, USA), 1.5 μ l of 10 pM primers, 6.5 μ l of PCR water (Sigma, St. Louis,

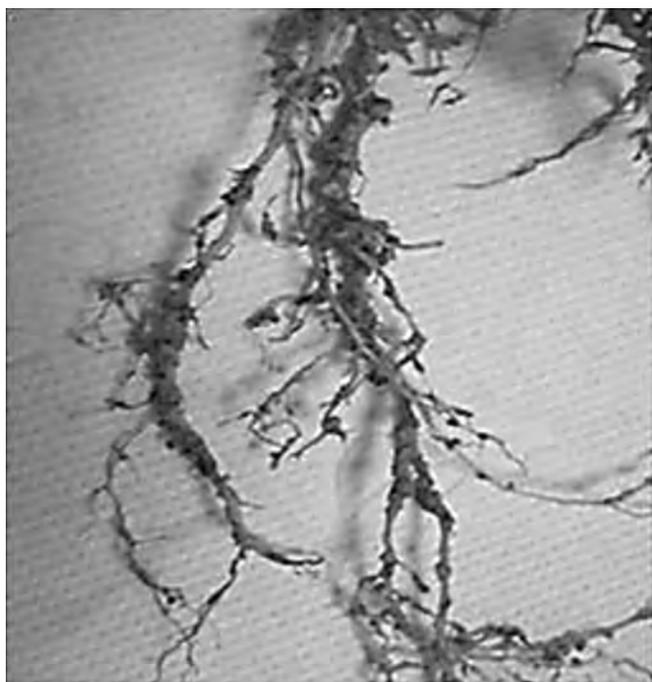


Fig. 2. Pepino (*Solanum muricatum*) roots infected with *Meloidogyne* spp.

MO, USA), and 2 μ l of genomic DNA.

The multiplex SCAR primers for *M. hapla* were JMV1, 5'-GGATGGCGTGCTTTCAAC-3', JMV2, 5'-TTTCCCCTTATGATGTTTACCC-3', and JMV hapla, 5'-AAAAATCCCCTCGAAAAATC CACC-3' (Wishart *et al.*, 2002). The primer sets for *M. arenaria* were Far, 5'-TCGGCGATAGAGGTAAATGAC-3' and Rar, 5'-TCGGCGATAGACTACAAACT-3' (Zijlstra *et al.*, 2000). The PCR was successfully amplified by 45 cycles: denaturation for 30 seconds at 94 °C, annealing for 30 seconds at 50 °C for *M. hapla*, 61 °C for *M. arenaria*, extension for 1 minute at 72 °C, with initial denaturation for 15 minutes at 94 °C and final extension of 10 minutes at 72 °C. PCR was performed using PTC-100, Bio-Rad.

RESULTS AND DISCUSSION

Identification of *Meloidogyne* species is important to design effective nematode management practices such as crop rotation and plant resistance (Hussey, 1990; Zijlstra 2000; Zijlstra and Van Hoof, 2006).

Based on morphological characters (Jepson, 1987; Karssen, 2002), esterase phenotypes and molecular analyses, the unknown *Meloidogyne* species were identified as *M. arenaria* and *M. hapla*. The perineal patterns of *M. hapla* were characterized by quite distinctive striae, which were broken, curved, twisted or curled around (Fig 3A). The vulva was sunken in an area variable in shape and devoid of striae. The perineal patterns of *M. arenaria* showed the characteristic low dorsal arch, which is indented near the lateral field and with irregular forks. Striae were smooth and slightly wavy (Fig. 4A). The morphology of the perineal patterns is considered the most important morphological character for species identification (Eisenback, 1985; Karssen and van Aelst, 2001).

An important tool that can be used for studying and diagnosing root-knot nematodes is the electrophoretic method (Esbenshade and Triantaphyllou, 1985). In this study, the esterase isozyme phenotype analysis of single young egg-laying females of *M. hapla* revealed a single esterase band (Fig. 3B). For *M. arenaria*, two distinct bands of esterase were observed as described by Esbenshade and Triantaphyllou (1985) (Fig. 4B). Esterases have been useful for differentiating the major *Meloidogyne* species (Esbenshade and Triantaphyllou, 1985; Cofcewicz *et al.*, 2004).

The *Meloidogyne* species from Ordu province was identified as *M. hapla* by the exact size of 440 bp amplicons by JMV *hapla* scar primer (Fig. 3C). Alkan (1962), Yüksel (1974), Elekçioğlu and Uygun (1994), Elekçioğlu *et al.*, (1994), Mennan and Ecevit (1996), Söğüt and Elekçioğlu (2000) reported that *M. hapla* is a rare species in Turkey, but Özarslandan *et al.* (2005) identified *M. hapla* on potato in Turkey. In addition, Diker (1959) observed the occurrence of this species in the Black Sea re-

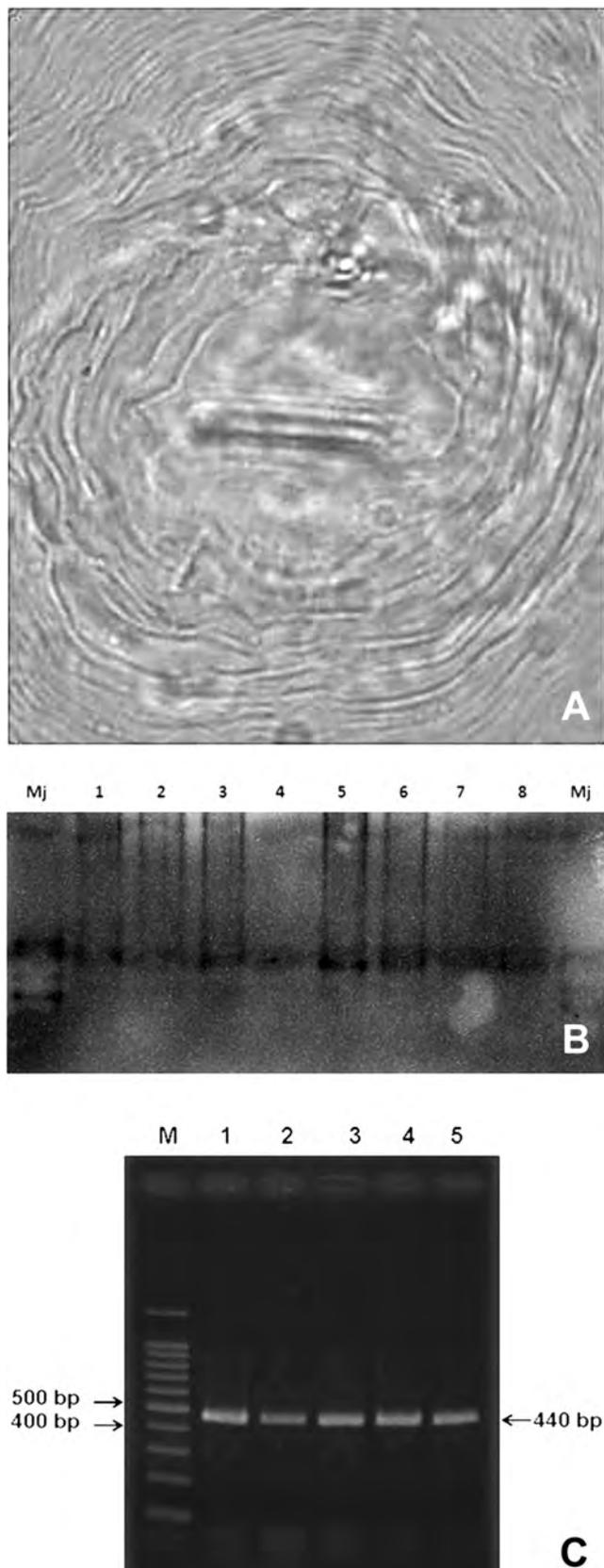


Fig. 3. *Meloidogyne hapla* from pepino at Ordu. A, perineal pattern of a female; B, esterase phenotypes on polyacrylamide gel. Lanes 1-8, one egg laying female. Left and right lanes *M. javanica* as a control; C, PCR-SCAR amplification. Lanes 1-4: Five females of *Meloidogyne* sp. each from Ordu. Lane 5, *M. hapla* as a control. M: GelPilot 100 bp ladder for molecular marker (QIAGEN).

gion. The other *Meloidogyne* species from Samsun province was identified as *M. arenaria* based on the size of the fragment, 420 bp, amplified by *M. arenaria* SCAR primer (Fig. 4 C). The multiplex SCAR primers that we used have been useful to differentiate *M. chitwoodi*, *M. fallax*, and *M. hapla* by the amplified sizes of the frag-

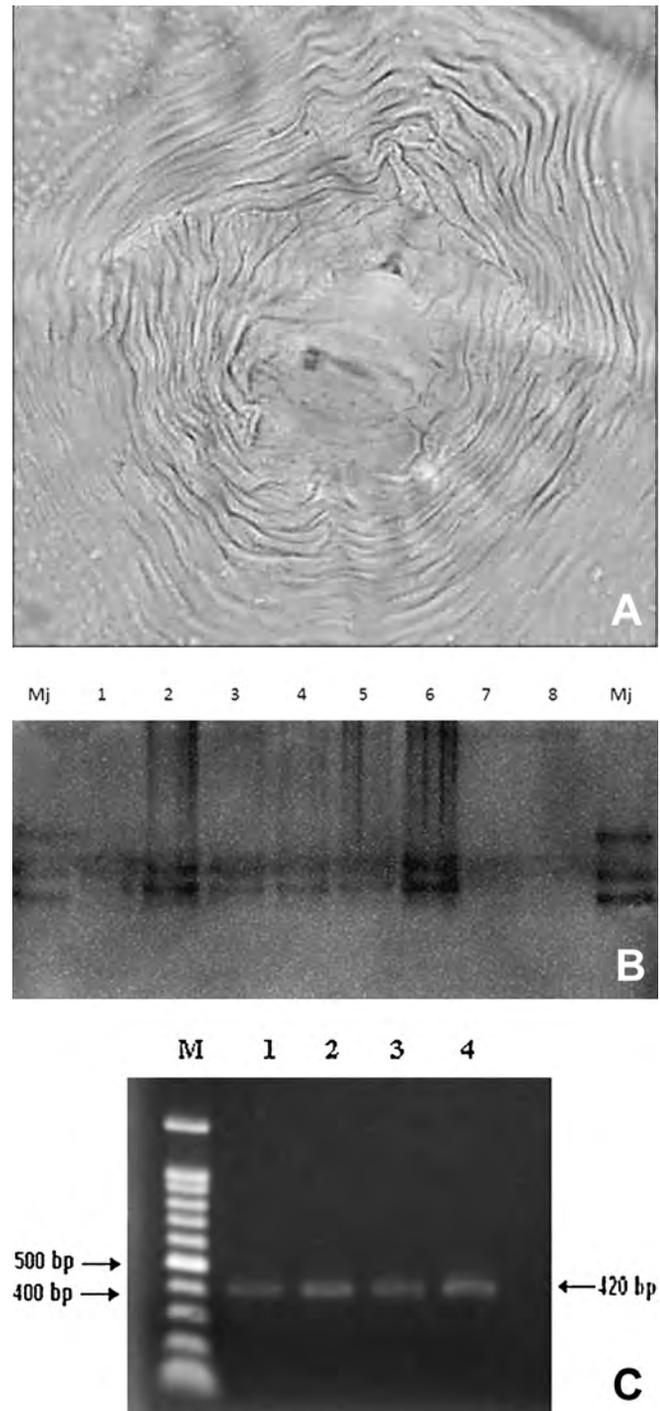


Fig. 4. *Meloidogyne arenaria* from pepino at Samsun. A, Perineal pattern of *M. arenaria* from pepino roots; B, Esterase phenotypes of the *M. arenaria* on polyacrylamide gel. Lanes 1-8, one egg laying female. Left and right lanes, *M. javanica* as a control; C, PCR-SCAR amplification of *M. arenaria* from Samsun. Lanes 1-3: Five females of *Meloidogyne* sp. each from Samsun. Lane 4: *M. arenaria* as a control. M: GelPilot 100 bp ladder for molecular marker (QIAGEN).

ments, 540 bp, 670, and 440 bp, respectively (Wishart *et al.*, 2002). Cucumber and tomato (Devran and Söğüt, 2009), eggplant (Özarslandan and Elekçioğlu, 2010), and parsley (Mennan *et al.*, 2011) were previously recorded as hosts for *M. arenaria* in Turkey.

To the best of our knowledge, this is the first report of *M. arenaria* and *M. hapla* infecting pepino in Turkey.

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LITERATURE CITED

- Alkan B., 1962. Türkiye'nin zararlı nematod faunası üzerinde ilk incelemeler. *Bitki Koruma Bülteni*, 2(12): 17-25.
- Cofcewicz E.T., Carneiro R.M.D.G., Castagnone-Sereno P. and Queneherve P., 2004. Enzyme phenotypes and genetic diversity of root-knot nematodes parasitizing *Musa* in Brazil. *Nematology*, 6: 85-95.
- Devran Z. and Sogut M.A., 2009. Distribution and identification of root-knot nematodes from Turkey. *Journal of Nematology*, 41: 128-133.
- Diker T., 1959. *Nemat parazit nematodları*. Türkiye Şeker Fabrikaları A.Ş. Neşriyatı, No:70, Ankara, Mars T.ve S.A.Ş. Matbaası, Türkiye, s. 98.
- Eisenback J.D., 1985. Diagnostic characters useful in the identification of the four most common species of root-knot nematodes (*Meloidogyne* spp.). Pp. 95-112. In: An Advanced Treatise on *Meloidogyne*, vol. I, Biology and Control (Sasser J.N. and Carter C.C., eds). North Carolina State University Graphics, Raleigh, USA.
- Elekçioğlu I.H. and Uygun N., 1994. Occurrence and distribution of plant parasitic nematodes in cash crop in Eastern Mediterranean region of Turkey. Proceedings of the 9th Congress of the Mediterranean Phytopathological Union, 18-24 September, 1994, Aydın, Turkey, pp. 409-410.
- Elekçioğlu I.H., Ohnesorge B., Lung G. and Uygun N., 1994. Plant parasitic nematodes in the Mediterranean region of Turkey. *Nematologia Mediterranea*, 22: 59-63.
- Esbenshade P.R. and Triantaphyllou A.C., 1985. Electrophoretic methods for the study of root-knot nematode enzymes. Pp. 115-123. In: An Advanced Treatise on *Meloidogyne*, vol II, Methodology (Barker KR, Carter C.C. and Sasser J.N., eds). North Carolina State University Graphics, Raleigh, USA.
- Harris H. and Hopkinson D.A., 1976. *Handbook of enzyme electrophoresis in human genetics*. North-Holland Publications, New York, USA, 200 pp.
- Hartman K.M. and Sasser J.N., 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal patterns morphology. Pp. 69-77. In: An Advanced Treatise on *Meloidogyne*, vol. II, Methodology (Barker K.R., Carter C.C. and Sasser J.N., eds). North Carolina State University Graphics, Raleigh, USA.
- Hussey R.S., 1990. Biochemical and molecular methods of identifying *Meloidogyne* species: symposium introduction. *Journal of Nematology*, 22: 8-9.
- Jepson S.B., 1987. *Identification of root-knot nematodes*. CABI, Wallingford, UK, 265 pp.
- Karszen G., 2002. *The plant-parasitic nematode genus Meloidogyne Goeldi, 1892 (Tylenchida) in Europe*. Brill, Leiden, The Netherlands, 157 pp.
- Karszen G. and van Aelst A.C., 2001. Root-knot nematode perineal pattern development: a reconsideration. *Nematology*, 3: 95-111.
- Knight K.W.L., Barber C.J. and Page G.D., 1997. Plant parasitic nematodes of New Zealand recorded by host association. *Journal of Nematology*, 29(4S): 640-656.
- Martinez-Romero D., Serrano M. and Valero D., 2003. Physiological changes in pepino (*Solanum muricatum* Ait.) fruit stored at chilling and non-chilling temperatures. *Postharvest Biology and Technology*, 30: 177-186.
- Mennan S. and Ecevit O., 1996. Bafra ve Çarşamba ovaları yazlık sebze ekim alanlarındaki kök-ur nematodları (*Meloidogyne* spp.)'nın biyolojisi, yayılışı ve bulaşık oranları üzerine araştırmalar. Proceedings of the Third Turkish National Congress of Entomology, 24-28 september 1996. Ankara, Turkey, pp. 700-705.
- Mennan S., Aydinli G. and Kati T., 2011. First report of root-knot nematode (*Meloidogyne arenaria*) infecting parsley in Turkey. *Journal of Phytopathology*, 159: 694-696.
- Özarslandan A., Söğüt M.A. and Elekçioğlu I.H., 2005. A new host of *Meloidogyne hapla* in Turkey. *Journal of The Faculty of Agriculture, Harran University*, 9(3): 63-64.
- Özarslandan A. and Elekçioğlu I.H., 2010. Identification of the root-knot nematode species (*Meloidogyne* spp.) (Nemata: Meloidogynidae) collected from different parts of Turkey by molecular and morphological methods. *Turkish Journal of Entomology*, 34: 323-335.
- Prohens J., Ruiz J.J. and Nuez F., 1996. The pepino (*Solanum muricatum*, Solanaceae): A new crop with a history. *Economic Botany*, 50: 355-368.
- Söğüt M.A. and Elekçioğlu I.H., 2000. Determination of *Meloidogyne* Goeldi, 1892 (Nemata: Heteroderidae) species races found in vegetables growing areas of the Mediterranean region of Turkey. *Turkish Journal of Entomology*, 24: 33-40.
- Trudgill D.L. and Blok V.C., 2001. Apomictic polyphagous root knot nematodes: exceptionally successful and damaging biotrophic root pathogens. *Annual Review of Phytopathology*, 39: 53-77.
- Wishart J., Philips M.S. and Blok V.C., 2002. Ribosomal intergenic spacer: a polymerase chain reaction diagnostic for *Meloidogyne chitwoodi*, *M. fallax*, and *M. hapla*. *Phytopathology*, 92: 884-92.
- Yüksel H., 1974. The distribution of root-knot nematodes (*Meloidogyne* spp.) in Turkey. *Ataturk University, Journal of the Faculty of Agriculture*, 5: 83-105.
- 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 on PCR assay. *Nematology*, 2: 847-883.
- Zijlstra C. and Van Hoof R.A., 2006. A multiplex real time polymerase chain reaction (TaqMan) assay for the simultaneous detection of *Meloidogyne chitwoodi* and *M. fallax*. *Phytopathology*, 96: 1255-1262.