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Molecular Studies on Root-Knot Nematodes in Protected Cultivations of Turkey

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ABSTRACT

Turkey is one of the most important agricultural producers in the world. Protected vegetables are widely cultivated in the different regions of Turkey due to its climatic condition. Root-knot nematodes (RKN) cause considerable yield losses in the protected vegetable growing locations of Turkey. They infect plant roots, causing the recession on plant growth by development of root-knot galls draining the plant's photosynthate and nutrients. While infected young plants may be lethal, grown plants mainly cause greater yield losses. Correspondingly, the infection by nematodes leads secondary infection resulting soil borne pathogens. Therefore, they cause considerable yield losses depending conditions occurring in fields of intensively protected cultivations. The purpose of this review is to address identification and investigation of genetic variation of the root-knot nematodes using molecular methods, and explanation of breeding programs currently carrying out for them on tomato in Turkey.

Keywords: Markers, protected cultivations, root-knot nematodes, tomato, Turkey
Abbreviations: **AFLP**, amplified fragment length polymorphism; **RAPD**, random amplified polymorphic DNA; **RKN**, root knot nematodes; **RFLP**, restriction fragment length polymorphism

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INTRODUCTION

Molecular identification of RKN

Turkey is one of the world's most important vegetable producers. Vegetables are produced both in open and protected fields in the country. Protected vegetables are grown in the Mediterranean and Aegean regions of Turkey due to its favorable climatic conditions (Devran and Söğüt 2009). There are many pathogens causing economic yield losses in protected vegetable growing areas. Root-knot nematodes have a very wide host range, and consequently are becoming one of the important pathogens in agricultural lands (Devran and Söğüt 2009). Root-knot nematodes are soil-borne pathogens and can cause dramatic symptoms on the host plant roots by feeding. As a result of nematode feeding, large galls or knots can form throughout the root system of infected plants. Since galled roots have limited ability to absorb and transport water and nutrients upward to the rest of the plant, heavy infected plant may die in stress conditions (Netscher and Sikora 1990).

In the world, more than 80 species of root knot nematode have been described (Siddiqi 2000). Root-knot nematode species have been identified on different crops in Turkey (Elekçioğlu and Uygun 1994; Elekçioğlu *et al.* 1994). Although some studies on the identification and management of root-knot nematodes had been done before, comprehensive works have been carried out in different regions of Turkey since 1990 (Elekçioğlu and Uygun 1994; Elekçioğlu *et al.* 1994; Mennan and Ecevit 1996; Kaşkavalcı and Öncüer 1999). Distributions rate of root-knot nematodes in processing tomato grown fields of İzmir, Manisa, Balıkesir, Bursa and Çanakkale provinces were studied in 1992-1993 years. Distribution of *Meloidogyne incognita* and *M. javanica* were 72.97 and 27.03%, respectively (Pehlivan and Kaşkavalcı 1992, 1993). The other study was carried out in Aydın province. *Meloidogyne incognita* (80.06%), *M. javanica* (14.49%) and *M. hapla* (5.45%) were determined in the province (Kaşkavalcı and Öncüer, 1998, 1999). In Black Sea region of Turkey, *M. incognita M. arenaria* and *M. hapla* were found as 57.2%, 33.3% and 9.5% in vegetable growing fields in Samsun, respectively (Katı and Mennan 2006).

Some studies were carried out on determination of root knot nematodes races in different regions of Turkey. Presence of *M. incognita* race 2 in vegetables growing areas of East Mediterranean (Adana and Mersin provinces) and Black Sea regions (Samsun province) was published (Söğüt and Elekçioğlu 2000; Mennan and Ecevit 2001). Devran and Söğüt (2011) also reported that *M. incognita* race 2, *M. javanica* race 1 and *M. arenaria* race 2 were present in the West Mediterranean region. Moreover, *M. incognita* race 6 and *M. arenaria* race 3 were reported as the first formal record on the new races of root knot nematodes in Turkey.

In Turkey, first molecular study on root-knot nematodes were carried out in 2002 (Devran *et al*. 2002). Comprehensive study on molecular identification of root-knot nematodes collected from protected areas (**Table 1**) has been conducted in the West Mediterranean region of Turkey and *M. incognita*, *M. javanica* and *M. arenaria* have been identified using species-specific primers (Devran and Söğüt 2009). *Meloidogyne incognita* was identified using three

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Invited Mini-Review

Table 1 Root-knot nematodes populations used in this study (Devran and Söğüt 2009, modified)

Code	Location	Host plant	Species	Code	Location	Host plant	Species
K10	Kumluca	Pepper	*M. incognita*	O1	Ortaca	Tomato	*M. arenaria*
K11	Kumluca	Tomato^R	*M. incognita*	O4	Ortaca	Tomato	*M. arenaria*
K12	Kumluca	Pepper	*M. incognita*	O6	Ortaca	Tomato	*M. arenaria*
K14	Kumluca	Tomato	*M. incognita*	O7	Ortaca	Tomato	*M. arenaria*
K15	Kumluca	Pepper	*M. incognita*	G1	Gazipaşa	Tomato	*M. javanica*
K17	Kumluca	Eggplant	*M. incognita*	G5	Gazipaşa	Eggplant	*M. javanica*
K19	Kumluca	Tomato	*M. incognita*	A1	Alanya	Eggplant^R	*M. javanica*
D1*	Demre	Tomato	*M. incognita*	A4	Alanya	Cucumber	*M. javanica*
D2*	Demre	Tomato	*M. incognita*	A5	Alanya	Tomato	*M. javanica*
D4	Demre	Tomato	*M. incognita*	A7	Alanya	Cucumber	*M. javanica*
D5	Demre	Tomato	*M. incognita*	AKS1	Aksu	Tomato	*M. javanica*
D6	Demre	Bean	*M. incognita*	AKS2*	Aksu	Tomato	*M. javanica*
D7	Demre	Bean	*M. incognita*	AKS3	Aksu	Tomato^R	*M. javanica*
KA1	Kaş	Tomato	*M. incognita*	AKS4*	Aksu	Tomato	*M. javanica*
KA2	Kaş	Pepper	*M. incognita*	AKS5	Aksu	Tomato	*M. javanica*
KA3	Kaş	Pepper	*M. incognita*	AKS6	Aksu	Tomato	*M. javanica*
KA4	Kaş	Pepper	*M. incognita*	AKS7	Aksu	Tomato	*M. javanica*
KA5	Kaş	Tomato^R	*M. incognita*	M7	Altınova	Tomato	*M. javanica*
KA7	Kaş	Tomato^R	*M. incognita*	M8	Altınova	Tomato	*M. javanica*
F1	Fethiye	Tomato	*M. incognita*	M11	Altınova	Tomato	*M. javanica*
F2	Fethiye	Tomato	*M. incognita*	K13	Finike	Tomato	*M. javanica*
F6*	Fethiye	Tomato	*M. incognita*	K16*	Finike	Tomato	*M. javanica*
F9	Fethiye	Tomato	*M. incognita*	K20	Finike	Cucumber	*M. javanica*
F10*	Fethiye	Tomato	*M. incognita*	K21	Finike	Cucumber	*M. javanica*
F11	Fethiye	Tomato	*M. incognita*	D3	Demre	Tomato	*M. javanica*
F12	Fethiye	Tomato	*M. incognita*	KA6	Kaş	Tomato^R	*M. javanica*
F13	Fethiye	Tomato	*M. incognita*	F4*	Fethiye	Tomato	*M. javanica*
F15	Fethiye	Tomato	*M. incognita*	F5*	Fethiye	Tomato	*M. javanica*
O2	Ortaca	Tomato	*M. incognita*	F7*	Fethiye	Tomato	*M. javanica*
O5	Ortaca	Tomato	*M. incognita*	F8	Fethiye	Tomato	*M. javanica*
M9	Altınova	Tomato	*M. arenaria*	F14	Fethiye	Tomato	*M. javanica*
K18	Kumluca	Tomato	*M. arenaria*	O3	Ortaca	Tomato	*M. javanica*
K22	Finike	Cucumber	*M. arenaria*				

Tomato^R: Tomato rootstock, Eggplant^R: Eggplant rootstock, * Virulent population

different primer sets including SEC-1F/SEC-1R, inc-K14-F/ inc-K14-R and MIF/MIR. PCR with SEC-1F/SEC-1R produced approximately 500 bp for *M. incognita* populations (**Fig. 1A**). *Meloidogyne javanica* was identified by Fjav-Rjav and DJF/DJR primer sets. The Fjav-Rjav primer set yielded 670 bp for *M. javanica* (**Fig. 1B**). PCR with Far/Rar primer set produced a 420 bp fragment, which was characteristic in populations of *M. arenaria* (**Fig. 1C**). Furthermore, *M. incognita* has been described as the most widespread species in protected vegetables growing areas of this region (Devran and Söğüt 2009). In other study, root-knot nematodes collected from many provinces of Turkey were identified by molecular methods (Özarslandan and Elekçioğlu 2010).

The knowledge of genetic variation inter/intra root-knot nematode species is of great importance especially in terms of resistance breeding. Different molecular markers including RFLP, RAPD, AFLP have been employed to evaluate population genetic variation in *Meloidogyne* spp. (Curran *et al*. 1996; Carneiro *et al*. 1998; Semblat *et al*. 1998). Genetic variation of root knot nematodes collected from vegetables growing areas in the Mediterranean region of Turkey were analyzed by AFLP marker system and reported that genetic intra-specific similarity of *M. arenaria M. incognita*, and *M. javanica* ranged from 40 to 67%, 45 to 56% and from 41 to 73%, respectively. These results also showed general correlation was not found between genomic similarity and geographical origin of the populations (Devran *et al*. 2008).

In conclusion, root-knot nematodes are important pathogens of protected vegetable growing aeras in Turkey. Therefore, the knowledge of intra/inter genetic variation in *Meloidogyne* spp., and rapid and accurate identification of root knot nematodes are important for pest management, improvement of resistance cultivars, and molecular screening of populations. These complicate issues are successfully performed by molecular techniques.

Marker-assisted selection for RKN in tomato

Tomato is one of the important vegetables grown in protected fields of Turkey. Root-knot nematodes cause significant yield losses in tomato production regions. Soil fumigants, contact–systemic nematicides and resistant varieties/rootstocks are commonly used for controlling root-knot nematodes in protected areas (Devran *et al*. 2010). Each of these methods has certain advantages depending on application areas and cultivation period. Chemical pesticides have been widely used for controlling root-knot nematodes. However, environmental effects and government regulations have drastically restricted the use of nematicides. Alternatives to nematicides are needed and resistant breeding is one of the most effective methods to control root-knot nematode. Resistant plants prevent completely reproduction of nematodes or sustain their population numbers fairly low, and they need no special application techniques or tools. Resistant plants are also cost effective and environmentally friendly according to susceptible plants (Cook and Evans 1987).

Root-knot nematode resistance in tomato is controlled by a single dominant gene, *Mi-1*. The gene confers resistance to three of the most damaging species of root knot nematodes including *M. incognita*, *M. javanica* and *M. arenaria*. Most available resistant commercial varieties bear this *Mi-1*. This gene was transferred from wild tomato *Solanum peruvianum* (PI. 128657) to cultivated tomato *Solanum esculentum* via embryo rescue (Smith 1944). *Mi-1* gene is located on sort arm of chromosome 6. Molecular markers linked to the gene have been identified and mapped (Messequer *et al*. 1991; Williamson *et al*. 1994). In another study, *Mi-1* gene has been cloned and isolated from resistant plant (Milligan *et al*. 1998).

The *Mi* gene has been successfully used for controlling of *M. incognita*, *M. javanica* and *M. arenaria*. However, this gene is irreversible inactive above 28°C soil temperature (Dropkin 1969) and fail to work in *Mi-1* virulent root-

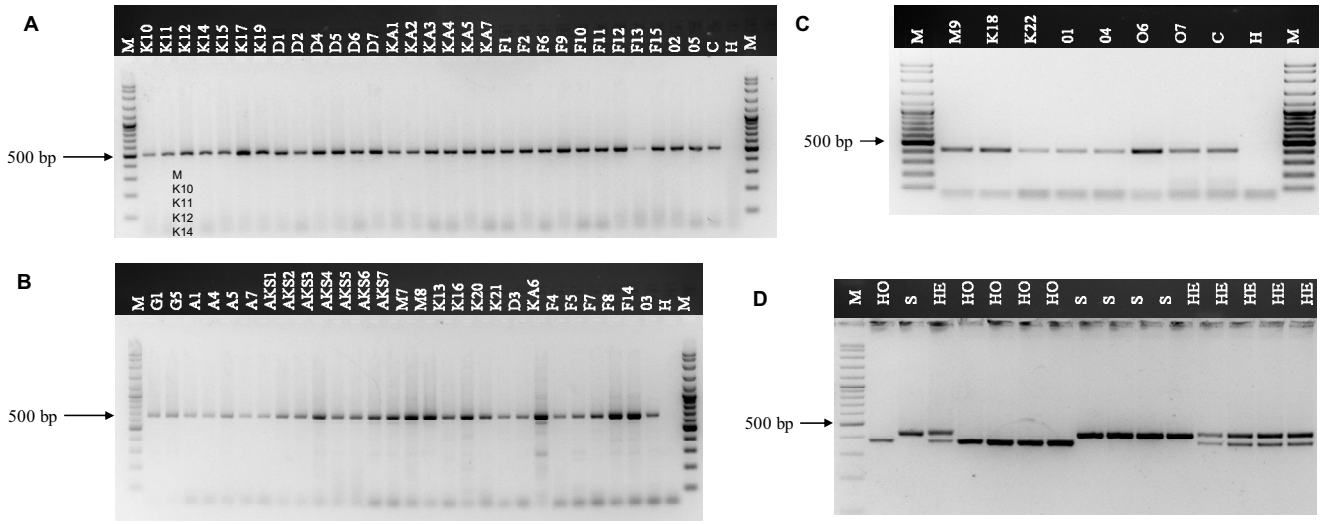

Fig. 1 (**A**) Amplification products with the *M. incognita* species-specific SEC-1F/SEC-1R primers. M: 100-bp DNA ladder (Vivantis); Samples (K10, K11, K12, K14, K15, K17, K19, D1, D2, D4, D5, D6, D7, KA1, KA2, KA3, KA4, KA5, KA7, F1, F2, F6, F9, F10, F11, F12, F13, F15, O2, O5), C: positive control, H: water. (**B**) PCR products with the *M. javanica* species-specific Fjav-Rjav primers. M: 100-bp DNA ladder (Vivantis), samples (G1, G5, A1, A4, A5, A7, AKS1, AKS2, AKS3, AKS4, AKS5, AKS6, AKS7, M7, M8, K13, K16, K20, K21, D3, KA6, F4, F5, F7, F8, F14, O3), H: water. (**C**) Amplification products with the *M. arenaria* species-specific Far-Rar primers. M: 100-bp DNA ladder (Vivantis); samples (M9, K18, K22, O1, O6, O7, C), H: water. (**D**) Amplification products of Mi23 marker. M: 100-bp DNA ladder (Vivantis), HO: homozygous plant, S: susceptible plant, HE: heterozygous plant. (**A-C**) Modified from Devran and Söğüt 2009; (**D**) modified from Devran *et al.* 2010.

knot nematode populations (Castagnone-Sereno 1994; Kaloshian *et al*. 1996). In the Mediterenean region of Turkey, occurrence of virulent root-knot nematode populations were investigated and results showed that seven populations of *M. incognita* and six populations of *M. javanica* overcame tomato plants carrying the *Mi-1* gene (Devran and Söğüt 2010).

Resistant breeding for root-knot nematode is very difficult and time-consuming compared to conventional methods. These difficulties can be overcome by using molecular markers in breeding programs. The use of molecular markers reduces both time and greenhouse space needed to develop a new resistant line or variety. These methods have been used in the selection of individuals carrying the *Mi-1* gene allele. Molecular marker called co-dominant also allows distinguishing whether the amplified DNA segment is heterozygous or homozygous (Williamson *et al*. 1994; El Mehrach *et al*. 2005; Seah *et al*. 2007; Arens *et al.* 2010). In our resistant breeding studies also, molecular markers linked to *Mi-1* gene have been effectively used, and successfully separated homozygous and heterozygous lines (**Fig. 1D**) (Devran and Elekçioğlu 2004; Devran *et al*. 2010).

In conclusion, for an effective molecular marker in marker-assisted selection, it must be an economic, robust and it has to have a rapid screening capacity. The molecular markers that are being used in resistance to root knot nematodes in tomato breeding have these properties.

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