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Selection of Root-Knot Nematod Resistance in Inbred Tomato Lines Using CAPS Molecular Markers

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Abstact: Tomato root-knot nematode, is one of the most important disease agents in tomatoes (Solanum lycopersicum). A practical and effective method for controlling this disease is by means of tomato genotypes with resistance genes against this disease. Tomato plants are offered resistance to root-knot nematode by Mi gene. Phenotypically observing genetic lines in terrestrial conditions is difficult and time consuming due to concerns about the transfer of this agent to other plants and their stability. By using the molecular markers related to resistance genes, this problem can be avoided and the selection efficiency can be increased. In this project, 34 inbred lines were tested using 1 CAPS marker associated with Mi gene, resistant and susceptible genotypes were identified. In the study, 34 genotypes were not found to be homozygous resistant (MI / MI) genotypes whereas 7 genotypes were found to be heterozygous resistant (MI / mi) and 27 genotypes (mi / mi)homozygous susceptible. As a result of this study, root-knot nematode is the basis for developing resistant varieties.

Key words: Tomato, Mi gene, CAPS

Introduction

Tomato (Solanum lycopersicum L.) is one of the most important horticultural crops worldwide [1] and a good source of vitamins A and C [2] widely grown around the world. According to the figures of the year 2014, tomatoes produced in the world with 5,023,810 hectares of land 170,750,767 tons were produced in 319,109 hectares and 11,850,000 tons in Turkey. With this amount, Turkey, comes in the 4th place after China and the USA.

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Root-knot nematodes (RKNs), *Meloidogyne* spp., are considered to be the most damaging nematode group in the world as they cause high yield losses to most cultivated plant species in subtropical and tropical regions (Chen [3, 4]. RKNs, *Meloidogyne* spp., are obligate, sedentary endoparasites of many plant species [5]. Their potential host range encompasses more than 3000 plant species [6]. The most economically important species are *M. arenaria Chitwood, M. javanica Chitwood* and *M. incognita Chitwood*. In Turkey, the Mediterranean region is the most important for protected and open field vegetables. *M. arenaria, M. javanica* and *M. incognita* are among the major economic problems facing crop production in this region (Elekcioglu [7, 8].

The tomato crop is seriously affected by over 200 diseases caused by pathogenic fungi, bacteria, viruses and nematodes therefore a major goal of modern tomato breeding programs is the development of cultivars with improved disease resistance [9]. Most of the current management strategies to control RKNs rely on genetic resistance, cultural control and nematicide application (Chen [3, 10]. The most practical and effective method to control root-knot nematode disease is host resistance against this disease because other applications such as the use of nematicides, the concerns about environmental and human health risks, and the high cost limits the use of nematists [6, 10, 11].

However, currently only the Mi gene confers resistance against root-knot nematodes and it was introgressed to Lycopersicon esculentum from its wild relative L. Peruvicanum in the early 1940s by embryo rescue [12]. In tomato, resistance to RKN is controlled by a single dominant gene designated as Mi-1 gene. Nowadays Mi-1 gene is a single source resistance to root-knot nematodes in cultivated cultured tomatoes. The Mi-1 gene confers resistance to three species of RKN, Meloidogyne incognita, Meloidogyne javanica and Meloidogyne arenaria (Roberts [13-15]. This tomato gene is mapped on 6. Chromosome. However, Mi-1 mediated resistance to RKNs can lose efficacy when soil temperatures are higher than 28 °C and can also be hampered by the occurrence of resistance-breaking populations [16].

After identifying the genes found in the genomic pathway associated with resistance to a disease, there are various methods for monitoring these genes, such as molecular markers. Genomic approaches can be enhanced by the identification of genes for resistance to disease and the identification of markers associated with these genes.

Material and Methods

In this study, 34 inbred lines of tomato (Lycopersicon esculentum Mill.) were used as plant material. Tomato seeds were germinated in a 1: 1 mixture of peat perlite in violel. The genomic DNA extraction was performed according to the manufacturer's protocol instructions (QIAGEN, Hilden Germany) using 100 mg tomato leaf tissue as starting material. A final volume of 50 µl Nuclease-free water was used to eluate DNA. The DNA concentration was determined by the absorbance at 230, 260 and 280 nm, using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, USD). And the final concentration of each sample was set at 10 ng µl -1.

In this study, PCR-based CAPS marker was used as DNA marker. Each PCR reaction contained a total of 4 µl master mix (1.5 mM MgCl2, 0.2 mM dNTPs, 0.5 Units Taq DNA polymerase and 10x PCR buffer) (Rosebio), 4 µl DNA (10 ng µl-1), 1 µl primers Forward and reverse primers (Centromere DNA Technologies) and 10 µl of nuclease-free water. PCR amplification was performed on a Biorad T100 thermal cycler according to the standard PCR method. Primer sequences used in PCR studies are given in Table 1. PCR conditions for Rex Primer were as follows: 1 cycle at 95 ° C for 3 minutes, 30 cycles at 95 ° C, 30 seconds at 52 ° C, 30 cycles at 72 ° C, 35 cycles at 95 ° C, 5 minutes. At the end of PCR amplification, PCR products were separated on a 1% (W / V) agarose gel and 0.5 µg ml -1 ethidium bromide was added to visualize DNA bands. The sizes of the DNA fragments were determined using a 50 bp DNA ladder (Biolab). In order to confirm PCR amplification and gel electrophoresis results, the study was repeated at least twice.

PCR products were cut with Taq 1 enzyme to distinguish genotypes as susceptible or resistant. In total, 7.5 µl of PCR product was added, and 0.5 µl of Taq 1 enzyme (BIOWORLD 4000 U / ml) and 1 µl of Cut start buffer were added and the PCR was allowed to stand at 65 ° C for 15 minutes in thermal cycler. After cleavage was complete, the fragments were run on 2% agarose gel electrophoresis at 80 volts for 3 hours. After gel electrophoresis, PCR products were imaged using a Syngene gel imaging device.

Table1. Root-Knot Nematode Related iCAPS markers and their properties

Primer Name	Primer sequence	Marker Type	Resistance band	Susceptability band	Reference

Rex-F	(5'-TCGGAGCCTTGGTCTGAATT-3'	SCAR			[17]
Rex-R	5'-GCCAGAGATGATTCGTGAGA-T	SCAR			

Results

In the study, a total of 34 inbred tomato lines were identified to contain Mi gene, which genetically provides resistance to root-knot Nematode. In CAPS marker studies with Rex1 primers, PCR products produced a single band at a size of 750 bp in 34 tomato lines used in the experiment when electrophoresed prior to cutting with Taq1 enzyme (Fig1 and Fig2).

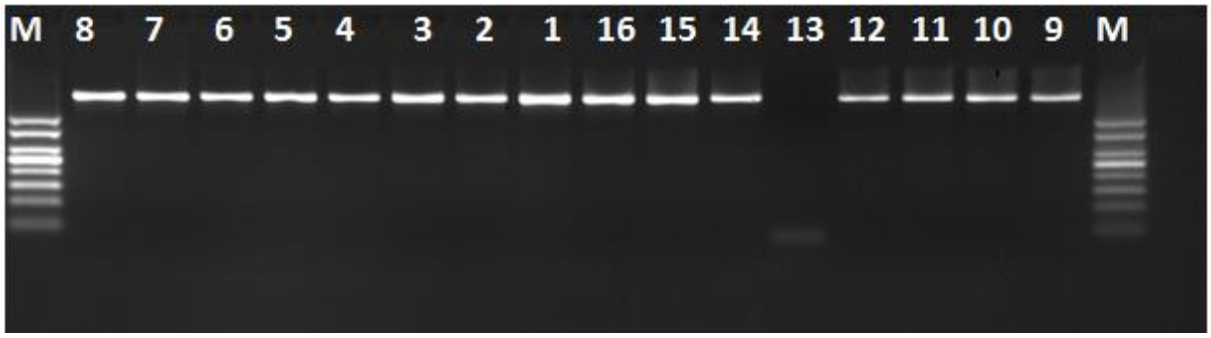

Fig 1. Amplification of tomato genotypes and CAPS markör by PCR in each genotype using the isolated DNA template. M: DNA marker (Biolab, 50 bp), 1 to 16 tomato genotype

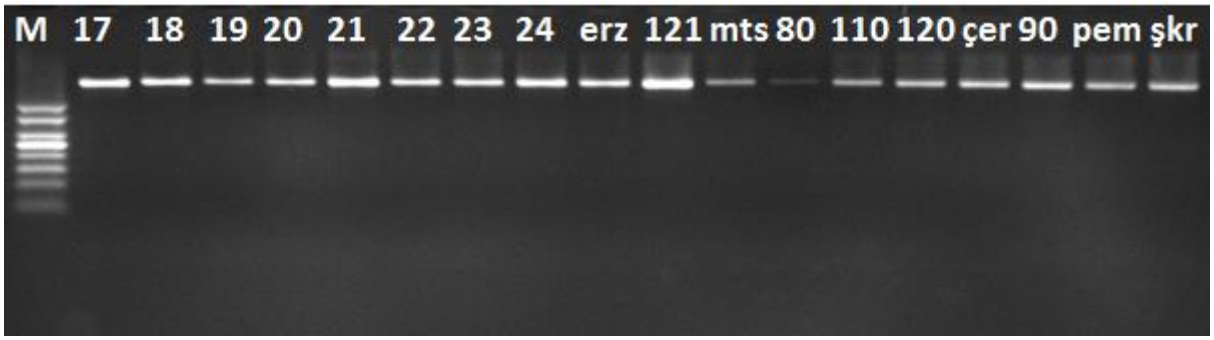

Fig 2. Amplification of tomato genotypes and CAPS markör by PCR in each genotype using the isolated DNA template. M: DNA marker (Biolab, 50 bp), 1 to şkr tomato genotype

PCR products with Rex primer resulted in 3 band profiles of 750, 570 and 160 bp after cutting with Taq1 enzyme (Fig. 3 and Fig. 4). The genotypes giving a single band at the size of 750 bp are homozygote-resistant (mi / mi), heterozygous for all three bands (Mi / mi), homozygous for genotypes giving 570 and 160 bp band. (Mi / Mi) (Williamson, Ho

et al., 1994, Duca, Port et al., 2012). As shown in Fig. 3 and Fig. 4, 34 genotypes produced mostly sensitive 750 bp fragments, while 1 genotype fragment was obtained and 7 gentipts produced 750 bp, 570 bp and 160 bp fragments. 15, 16, 20, 120, 110, 121, 90 were heterozygous, while other genotypes were susceptible. With this marker, the identification of homozygous and heterozygous resistant and susceptible tomato genotypes has been successfully accomplished. The results of these studies are similar in literature. The gel images obtained are shown in Fig. 3 and Fig.

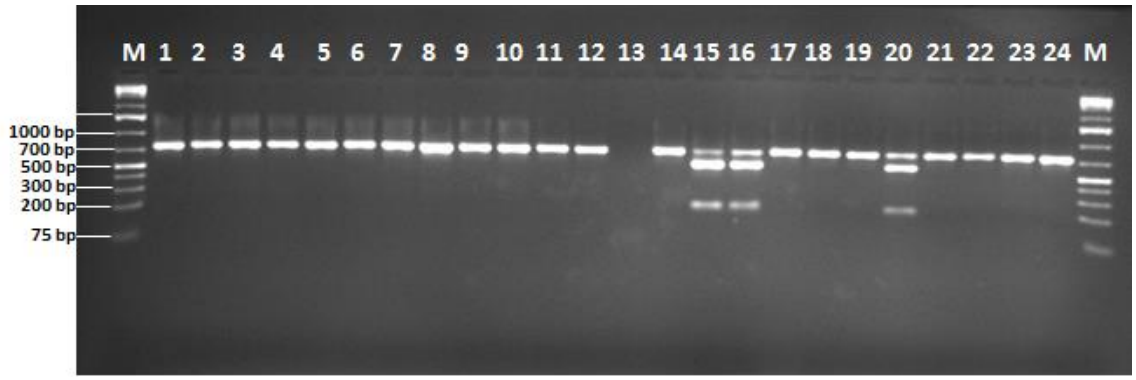

Fig. 3. Agarose gel image of 2% of Taq 1 enzyme cut-off results of tomato genotypes

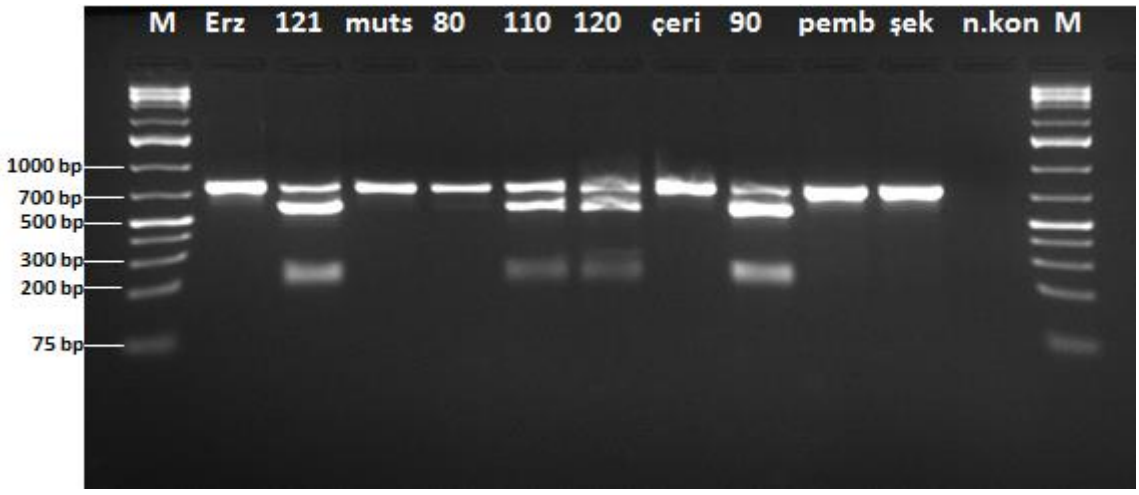

Fig. 4 Agarose gel image of 2% of Taq 1 enzyme cut-off results of tomato genotypes

Discussion and Conclusions

In disease control, the use of plants resistant to diseases is preferred due to the environmental risks of chemical combat methods. The tomato gene Mi-1 confers

resistance to several of the most damaging root-knot nematode species and is widely deployed for nematode control [5]

Molecular markers have many applications in plant breeding and are commonly used to improve resistant varieties. Therefore, the development of confident molecular markers for resistance genes could be advantageous for rapid screening of breeding lines. Reliable molecular markers linked to Mi-1 gene have been developed in tomato because of difficulties in testing for RKNs.

These root knots alter the uptake of water and nutrients and interfere with the translocation of minerals and photosynthates in the host resulting in plants with poor yield, stunted growth, wilting, and susceptibility to other pathogens. Selection with assistance of Molecular markers does not only improve the efficiency of selecting resistant plants but also enhances efforts to combine nematode resistance in tomatoes.

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