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Determination of Resistance Status of Qualified Tomato Genotypes to *Meloidogyne incognita*, *Tomato spotted wilt virus*, *Tomato mosaic virus*, Verticillium Wilt

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is one of the most cultivated vegetables both in Türkiye and in the world. Türkiye is the 4th tomato producing country after China, India and the USA. According to consumer demands, tomato varieties such as pink, beef, bunch, cocktail are grown. The main goals in breeding are high yield, quality and resistance to stress factors, and molecular marker-assisted selection is a fast and reliable method, especially in determining the presence or absence of resistance genes to biotic stress factors. In this study, the resistance genes determined for 339 advanced tomato lines, which have the potential to become parent lines due to their agro morphological characteristics and disease resistance against *Meloidogyne incognita* (Root-knot nematode), *Tomato spotted wilt virus* (TSWV), *Tomato mosaic virus* (ToMV) and Verticillium wilt in the plant material. 235 were homozygous resistant to root-knot nematode, 172 homozygous resistant to Verticillium wilt, 201 homozygous resistant to TSWV and 211 homozygous resistant to ToMV were determined. It has been seen that breeding programs can be created with the results obtained.

Keywords: *Meloidogyne incognita*, Tomatoes, ToMV, TSWV, Verticillium wilt

Introduction

In Türkiye, tomato is an economically important crop and it faces many diseases and pests in terms of cultivation. These diseases and pests cause a loss of yield and quality in tomato production. To increase tomato production and obtain quality products, it is of very important to protect against diseases and pests in tomato cultivation. It is possible to gain an initial resistance advantage by selecting tomato varieties that are resistant to diseases and pests. This increases the plant's natural ability to cope with diseases and makes them less susceptible to pests. Breeding programs are carried out using genetic resources to increase resistance to various diseases and pests. As a result of these studies, more than 50 disease and pest-resistance genes have been identified in tomato and these genes are used in the development of commercial varieties (Bai and Lindhout, 2007).

Root-knot nematodes (*Meloidogyne* spp.) are an important pest that can cause serious economic losses in agricultural production. Root-knot nematodes are one of the species of plant parasitic nematodes that live in the soil. These nematodes colonize the roots of plants and inhibit the growth of the plant while feeding. Root-knot nematodes disrupting the plant's uptake of nutrient and water by forming tumor-shaped nodules on the roots of the plant. This situation negatively affects the development of the plant and causes weakening of the roots, yellowing, wilting and in general loss of yield and quality of the plant (Agrios, 2005). Root-knot

nematodes can reduce vegetable production by 50% to 80% (Siddiqi, 2000). The *Mi-1.2* molecular marker is linked to the *Mi-23* gene of *S. lycopersicum* is known to be a marker used to determine nematode resistance in plants, *Mi-23* marker of *S. lycopersicum* about 450 bp is amplified in susceptible plants of (mi/mi). In addition, it is stated that a 400 bp fragment is seen in resistant phenotypes (Mi/Mi) and both fragments are seen in heterozygous plants (Mi/mi) (Pérez-Almeida et al., 2016). *Meloidogyne incognita* resistance to the breed is provided by the *Mi* gene. This gene family includes subtypes such as *Mi-1.1*, *Mi-1.2* and *Mi-1.3* (Milligan et al., 1998). These genes are important genetic resources used in the development of varieties resistant to root-knot nematodes in tomato cultivation.

TSWV (*Tomato spotted wilt virus*) is a viral disease that is common in the tomato plant that causes severe economic losses. TSWV infection can cause crop losses of up to 60% or even 100% in tomato growing areas. (Roselló et al., 1996). Symptoms due to TSWV infection in genotype during development period in which the plant was in when the infection occurred (Moriones et al., 1998; Chaisuekul et al., 2003) may vary depending on the virus isolate and environmental conditions (Kaminska, 1993; Mitidieri et al., 2000). These factors can affect the symptoms and severity of the disease and determine how TSWV infection will appear in tomato plants. There are genes for resistance to TSWV in the tomato plant. These resistance genes make plants more resistant to TSWV infection in *Solanum peruvianum.* The *Sw-5* gene found in the genome is a dominant resistance gene to TSWV. This gene has been reported as an effective source of resistance to TSWV. The *Sw-5* gene may be effective against various isolates of TSWV and is considered a non-race-specific resistance gene. That is, it can be generally effective against different TSWV isolates (Stevens et al., 1991).

Tomato mosaic virus (ToMV) is a virus that causes viral disease in tomato plants. Symptoms associated with ToMV infection can vary among plants and cultivars, but generally include stunting, curling of leaves, mosaic patterns and mottling, necrosis, and textural yellowing (Ullah et al., 2017). *S. lycopersicum* resistance to ToMV (*Tomato mosaic virus*) in cultivated tomato plants is usually *S. habrochites,* it is provided by genes transferred from the wild species (Lee et al., 2015). Genes known as *Tm-1*, *Tm-2* and *Tm-2*^{2} play an important role in conferring resistance to ToMV in tomatoes. These genes limit or inhibit the infection of the virus in tomato by genetic resistance mechanisms. The *Tm-1* gene is known to be a resistance gene transferred from *S. habrochites* to *S. lycopersicum.* This gene has been used in the development of tomato varieties that are resistant to ToMV. If the *Tm-2* gene of *S. habrochites* is transferred, it has been reported that there is another resistance gene that has been passed down from some populations. The *Tm-2* gene works by a mechanism of action that limits the spread of ToMV. Finally, the *Tm-2*^{2} gene has been described as a genetic variant that has been introduced as a supplement to the *Tm-2* gene to confer stronger resistance to ToMV (Weber et al., 2004; Labate et al., 2007).

Verticillium wilt rarely causes death in the plant, but there is a important loss of yield due to the drying of all the lower leaves (Pegg and Brady, 2002). Resistance to Verticillium wilt disease is known to be a genetically controlled trait in some plant species. On the tomato plant *Verticillium dahliae* Kleb resistance to race 1 is controlled by a single dominant gene (*Ve*) located on the short arm of chromosome 9 of the plant. This resistance gene makes the plant resistant to Verticillium wilt disease (Simko et al., 2004).

In this study, it was aimed to determine the resistance levels of qualified tomato pure lines at different stages against *Meloidogyne incognita* (root-knot nematode), Verticillium wilt (Ve), *Tomato spotted wilt virus* (TSWV), *Tomato mosaic virus* (ToMV) by molecular methods and to show the usability of these materials in breeding programs.

Materials and Methods

In the study carried out in cooperation with the public-private sector, 339 tomato lines selected from the gene accession of Selko R&D company constituted the plant material of the study. In the study, the resistance levels of lines to root-knot nematode, *Tomato spotted wilt virus* (TSWV), *Tomato mosaic virus* (ToMV), Verticillium wilt were determined using DNA markers. 2 markers were used for the selection of root-knot nematode hardiness status (Table 1). The REX-1 and *Mi23* markers successfully distinguish between nematode-resistant and susceptible genotypes (Bhavana et al., 2019). The presence of resistant *Tm* genes was identified for the first time using sequence-characterized amplified region (SCAR) and allele-specific (AS1) markers for the *Tm-1* and *Tm-2* genes, respectively (Ashwini and Nagaraju, 2022). Several PCR-based markers related to the resistance gene have been reported (Kawchuk et al., 1994; Kawchuk et al., 1998; Kawchuk et al., 2001; Acciarri et al., 2007; Park et al., 2008). SCAR and SNP markers have been reported to be related to this durability (Kawchuk et al., 1998; Kawchuk et al., 2001). Markers developed to accurately and quickly screen for resistance to *Tomato spotted wilt virus* (TSWV) disease are designed to

identify different allele combinations of the *Sw-5* gene (Stevens et al., 1995; Chagué et al., 1996; Folkertsma et al., 1999; Śmiech et al., 2000).

DNA isolation was performed in tissues taken from healthy leaf samples for molecular characterization. The work on the effective fragmentation of samples for DNA extraction and the release of DNA from cells was carried out in the Qiagen Tissue Lyzer II device. The SCAR and CAPS marker portions of DNA samples obtained from tomato leaves are indicated (Table 1). PCR mix Liu et al. (1995) it has been prepared according to the protocol. SCAR and CAPS marker ranges were visualized by the Qiaxcel Fragment Analyzer (Qiagen Sample & Assay Technologies) capillary electrophoresis system and agarose gel electrophoresis.

Results and Discussion

To study the different genes that are reported to confer resistance to different diseases and pests, each (Maurya et al., 2023), primer pair specifically designed for the gene was used. Marker fragments were imaged with the capillary electrophoresis system, an analytical method in which DNA and other molecules move in an electric field. Out of all *Meloidogyne incognita* (Root-Knot Nematode) genotypes 235 homozygous (RR) and 79 heterozygous (Rr) genotypes were identified. Maurya et al. (2023) scan at the *Mi23* locus using the *Mi1.2* SCAR marker revealed that 5 plants carried a resistance allele in the homozygous state, and 6 plants reported no resistance alleles. Bhavana et al. (2019) REX-1 and *Mi23* markers successfully differentiated between nematode-resistant and susceptible genotypes. In addition, *Mi23* did not require restriction enzyme analysis and separated homozygous/heterozygous resistance sources.

211 homozygous (RR) and 76 heterozygous (Rr) genotypes were obtained against *Tomato mosaic virus* (ToMV). In the greenhouse study conducted by Ashwini and Nagaraju (2022) on ToMV, a total of 35 tomatoes were screened to determine the source of resistance by mechanical inoculation, and 6 were determined as hardy, 11 as moderately resistant, 12 as moderately susceptible, and 6 as susceptible.

172 homozygous (RR) and 78 heterozygous (Rr) genotypes were identified against Verticillium wilt. In their study, Kiymaci et al. (2023) reported that out of 70 tomatoes, 45 RR (homozygous resistant), 15 Rr (heterozygous) and 10 RR (susceptible) were found against Verticillium wilt. 201 homozygous (RR) and 75 heterozygous (Rr) genotypes were obtained against *Tomato spotted wilt virus* (TSWV). Pinar et al. (2013) tested 92 F_2 tomato populations with the *Sw-5.2* SCAR marker and 26 of them reported that they obtained a durability band at 550 bp and reported that 23 of them obtained a sensitivity band at 500 bp. Table 2 shows the resistance levels of *Meloidogyne incognita*, *Tomato mosaic virus*, Verticillium wilt, *Tomato spotted wilt virus* against diseases and pests.

Conclusions

In tomato cultivation, the presence of genotypes with disease and pest-resistance genes is of very important. These genotypes make plants resistant to diseases and make them less susceptible to pests. Diseases and pests can lead to a loss of yield and a decrease in quality in tomato production. Therefore, the development of genotypes with resistance genes is of great importance for tomato cultivation. Breeding studies are conducted to identify genotypes with resistance genes, to transfer these genes to more resistant genotypes, and eventually to produce more resistant tomato varieties. When the results of the study were examined, 235 lines resistant to Root-Knot-Nematode, 172 lines resistant to Verticillium wilt, 201 lines resistant to TSWV and 211 lines resistant to ToMV were determined among 339 genotypes. As a result, 339 tomato genotypes with known agro morphological structures at different levels of stability were determined to be homozygous and heterozygous for 4 different pathogen species.

In accordance with the data obtained, a valuable gene accession has been created with genes resistant to disease factors. It will allow us to obtain genotypes that are advantageous in terms of disease resistance in future tomato breeding studies. In this direction, the development of more resistant varieties for tomato varieties will contribute to the adoption of a more sustainable approach to diseases and pests.

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Table 1. Primers used to determine the level of resistance of genotypes to specified diseases.

Pathogen Name	Marker Name	Gene	Primer Forward	Primer Reverse	Reference
Meloidogyne incognita	SCAR	*MI-REX*	TCGGAGCCTTGGTCTG AATT	GCCAGAGATGATTCGTGA GA	(Garcia et al., 2007)
	SCAR	*Mi23*	TGGAAAAATGTTGAAT TTCTTTTG	GCATACTATATGGCTTGTT TACCC	
Tomato mosaic virus (ToMV)	SCAR	*Tm-2*	CACCTTTCCCTCTCCAA	CACCTTTCCCCTAAAGC	(Sobir et al., 2000)
Verticillium wilt	SCAR	*V2LeO3*	CAAACATAGCTGGAAG AATC	TAGGAGGAAAAGAATTGG	(Acciarri et al., 2007)
Tomato spotted wilt virus (TSWV)	SCAR	*Sw-5–2*	AATTAGGTTCTTGAAGC CCATCT	TTCCGCATCAGCCAATAG TGT	(Dianese et al., 2010)

Marker amplicon sizes: *MI-REX,* Durable: tapes digested with TaqI of 570 and 160 bp, Susceptible: remains uncleared (750 bp); *Mi23*, Rugged: 380 bp, Susceptible: 420 bp; *Tm-2*, Durable: 574 bp, Precision: 534 bp; *V2LeO3,* Resistant: HincII digested 428 and 601 bp fragments, Susceptible: remains uncleared (1029 bp); *Sw-5-2,* Resistive: 574 bp, Susceptible: 464 bp; *TY-1*, Resistant: TaqI-digested 500, 300 and 160 bp bands, Susceptible: TaqI-digested 500, 300 and 200 bp bands; *TY-2*, Resistive: 120 bp, Susceptible: 213 bp; *TY-3,* Resistant: 630 bp, Susceptible: 320 bp. The markers found in the relevant genes are: *MI-REX, Mi23, TY-2, Sw-5-2, I3* and *V2LeO3*

Table 2. Resistance status of *Meloidogyne incognita*, *Tomato spotted wilt virus*, *Tomato mosaic virus*, Verticillium Wilt.

Disease and Pest	Homozygote (RR)	Heterozygote (Rr)
Meloidogyne incognita	235 genotypes	79 genotypes
Tomato mosaic virus (ToMV)	211 genotypes	76 genotypes
Verticillium wilt	172 genotypes	78 genotypes
Tomato spotted wilt virus (TSWV)	201 genotypes	75 genotypes

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