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Determination of Resistance Levels of Selected Tomato Genotypes to *Meloidogyne incognita, Tomato Yellow Leaf Curling Virus* (TYLCV) *Verticillium Wilt, Fusarium oxysporum radicis, Fusarium Wilt, Tomato Spotted Wilt Virus* (TSWV)

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HIGHLIGHTS

- In our study, C1-3, C1-13, C1-21, C1-23, C1-24, C1-25, C1-34, C1-35, C1-36, C1-37, C1-39, C1-40, C1 Genotypes -42, C1-43, C1-46, C1-48, C1-56, C1-57, C1-59, C1-66, C1-67 showed homozygous resistance to all diseases and pests mentioned. It has formed the most important output of our work.

Abstract

Tomato is one of the most cultivated vegetables in the world. In this context, intensive tomato breeding studies are carried out around the world and new cultivars are emerging every day, which leads to great competition. In particular, resistance or tolerance levelstolerance levels to some important diseases and pests are considered important in cultivar breeding and in determining the commercial value of cultivars. In this context, the determination of resistance levels to 70 tomatoes, *Meloidogyne incognita, Tomato Yellow leaf curling virus* (Tylcv), *Verticillium wilt, Fusarium oxysporum radicis, Tomato spotted wilt virus* (TSWV), *Fusarium Wilt*, which have the potential to become parent lines at S8 level due to their agro-morphological characteristics formed the subject of this study. When the results of the study are examined, tomato genotypes showed resistance/sensitive levels according to combinations of alleles as 58 genotypes of RR (homozygous resistant), 10 Rr (heterozygous), 2 rr(sensitive) to *Meloidogyne incognita*, 45 RR (homozygous resistant), 15 Rr (heterozygous),10 rr (sensitive)to *Verticillium dahliae*, 10 to, 52 RR (homozygous resistant), 13 Rr (heterozygous), 5 rr (sensitive) to *Tomato Spotted Wilt Virus*,46 RR (homozygous resistant) 18 Rr (heterozygous), 6 rr (sensitive) to *Tomato Yellow leaf Curl Virus*, *Fusarium oxysporum (Fusarium wilt)* 49 RR (homozygous resistant), 13 Rr (heterozygous), 8 rr (sensitive), *Fusarium oxysporum radicis* (Frl) 52 Their resistances were determined as RR (homozygous resistant), 12 Rr (heterozygous), 6 rr (sensitive).

Keywords: Domates, *Meloidogyne incognita*, *Verticillium dahlia*, *Tomato Spotted Wilt Virus*, *Tomato Yellow leaf Curl Virus*, *Fusarium oxysporum (Fusarium wilt)*

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1. Introduction

It is reported that tomato is susceptible to many pathogens that limit or completely eliminate their growth and development (Klee and Giovannoni 2011). While viral diseases cause 80-100% losses undercover in tomato cultivation (Ates et al. 2019)other biotic stress factors are reported to cause 20-40% yield losses. While various methods such as the use of chemical pesticides and cultural practices are used in the fight against diseases and pests, especially the use of resistant varieties (Hull 2009), chemical control produces no results in the fight against viruses (Jones 2006). In the struggle to reduce the effects of biotic stress factors, as a result of unconscious and uncontrolled use of chemical pesticides, the resistance to diseases and pests can increase, and human health and the environment are adversely affected. The most effective, EcoReco-friendly economical method in the fight against the abovementioned diseases and pests is to use resistant varieties. The use of resistant varieties provides not only an increase in yield and quality but also a great decrease in the use of chemicals (Qi et al. 2022). Considering these reasons, it has become a necessity to use resistant varieties during the fight against viral diseases apart from other biotic stress factors (Erkan, 2020a). With this aspect, developing resistant varieties is among the important issues of plant breeding. In particular, adverse conditions such as viruses, bacteria, nematodes and fungi that cause biotic stresses limit tomato cultivation and cause significant yield losses (Grube et al. 2000). Root-knot nematodes (*Meloidogyne spp*.) are one of the main pests of tomatoes. Resistance to the strain *Meloidogyne incognita* was conferred by the Mi gene transferred from *Solanum peruvianum* (Smith 1944). Mi-1.1, Mi-1.2 and Mi-1.3 genes have been reported in the Mi locus (Milligan et al., 1998). *Tomato yellow leaf curling virus* (TYLCV) is a viral disease that does not have a chemical control method and causes high yield losses. Several resistant genes against TYLCV have been identified in wild species including *S. chilense* (Ty-1, Ty-3, Ty-4, and Ty-6), *S. habrochaites*(Ty-2), and *S. peruvianum* (ty-5). Ty1/Ty-3 and Ty-2 genes were successfully used for breeding new tomato varieties. Ty-1 and Ty-3 are mapped to chromosome 6 and Ty-2 is mapped to chromosome 11 and marker assisted selection procedures were implemented in breeding programs (Gill et al. 2019; Ji et al. 2007; Yang et al. 2014). Another viral disease that limits tomato cultivation is the *tomato spotted wilt virus* (TSWV). The presence of 8 TSWV resistance genes was determined in tomato. The Sw-5 gene, which is dominant against TSWV in the *S. peruvianum*genome, has been reported as a source of resistance to TSWV as it is not race-specific (Stevens et al., 1991). *Fusarium oxysporum f. sp. lycopersici* (FOL) and *Fusarium oxysporum f. sp.* the pathogen radicis lycopersici (FORL) is one of the root diseases that limit cultivation and cause yield losses in tomato-growing countries around the world, including Turkey (Cucu et al. 2020). Resistance to FORL in tomato is controlled by the Frl dominant gene (Roberts et al., 2000). *Verticillium wilt* (Ve) is one of the most important soil-borne fungal factors encountered in tomato cultivation (Song et al. 2017). Independent Ve1 and Ve2 genes providing pathogen resistance have been identified (Kawchuk et al. 2001). As in the whole world, intensive studies are carried out on tomato cultivation in Turkey, and many new varieties with commercial importance are coming to the market day by day, and it is a necessity to come up with new varieties in a competitive environment. In this study, the resistance of pure S8 level tomato lines against *Meloidogyne incognita, Tomato yellow leafroll virus* (TYLCV), *Verticillium wilt, Fusarium oxysporum radicis, Tomato spotted wilt virus* (TSWV), *Fusarium Wilt* was investigated by molecular methods. It is aimed to determine the use of these materials in breeding programs and to reveal the usability of these materials.

2. Materials and Methods

The study was carried out in cooperation with the private sector. 70 genetic materials obtained by the expansion of commercial varieties available in the market and reaching the S8 level by the preliminary selection, constituted the plant material of the study. The set of tomato genotypes was selected for resistance to *Meloidogyne incognita, Tomato Yellow leaf curling virus* (TYLCV), *Verticillium wilt, Fusarium oxysporum* radicis, *Tomato spotted wilt virus* (TSWV) and *Fusarium Wilt*. Two markers were used for the selection of *M. incognita*

resistance status (Table 1) (Garcia et al., 2007). TYLCV resistance was determined based on carrying either Ty-1/Ty-3 (Ji et al., 2007; Barbieri et al., 2010), or Ty-2 (Yang et al., 2014; Kim et al., 2020) resistance alleles. Selection for Verticillium wilt was performed using the CAPS marker described by Acciarri et al. (2007), *Fusarium oxysporum* f. sp. *radicis*resistance was determined according to Mutlu et al. (2015), TSWV resistance was monitored using the SCAR marker introduced by Dianese et al. (2010) and Fusarium wilt resistance was evaluated based on a CAPS (Staniaszek et al. 2007) and a SCAR marker (Zhang and Panthee, 2021) (Table 1). For molecular characterization, leaf samples were taken for DNA isolation from each genotype from healthy, young leaves of plants in the young seedling stage. Leaf tissue samples were stored at -20 °C. Tissue homogenization for DNA extraction was performed using a Qiagen Tissue Lyzer II device. SCAR and CAPS marker fragments (listed in Table 1) amplified from tomato leaf DNA samples. PCR mixtures of 20µL were prepared as follows: 0.5 unit Amplitaq Gold® polymerase, 1x AmplitaqGold® PCR Buffer, 2.5 mM $MgCl_2$, 200µM each dNTP, 300 nM each primer,1.0µL of template DNA (concentration adjusted to 50 ng/µL)and nuclease-free H_2O. PCR cycling conditions were as follows: 10 min/ at 95°C initial denaturation, 35 cycles of 30 sec/95°C denaturation; 30 sec/60°C annealing reaction, 30 sec/72°C extension, followed by a final extension step of 10 min/72°C Restriction reactions for CAPS markers; Restriction reactions were prepared in 20µl mixtures containing 10U restriction enzyme (NEB), 1X restriction buffer, 5µl PCR product and distilled, deionized water. SCAR and CAPs marker fragments were visualized with the Qiaxcel Fragment Analyzer (Qiagen Sample & Assay Technologies) capillary electrophoresis system and agarose gel electrophoresis.

3. Results

Since different genes provide resistance to diseases, different primer pairs were screened for each gene. Marker fragments were visualized with a capillary electrophoresis system. As a result, 58 (82%) homozygous, 10 (14%) heterozygous and 2 (2%) sensitive resistance to *Meloidogyne incognita* were determined.45 (64%) homozygous, 15 (21%) heterozygous and 10 (10%) sensitive resistance to Verticillium wilt were determined. 52 (74%) homozygous, 13 (18%) heterozygous and 5 (5%) sensitive resistance to Tomato Spotted Wilt Virus were determined. 46 (65%) homozygous, 18 (25%) heterozygous, 6 (8%) sensitive resistance to Tomato Yellow leaf Curl Virus were determined. To *Fusarium oxysporum* (*Fusarium wilt*) 49 (70%) homozygous, 13 (18%) Rr heterozygote, 8 (11%) sensitive resistance levels were determined. *Fusarium oxysporum radicis* (Frl) 52 (74%) homozygous resistance, 12 (17%) heterozygous resistance, and 6 (7%) sensitive resistance levels were determined. In Table 2, resistance to diseases and pests of *Meloidogyne incognita, Verticillium dahlia, Tomato Spotted Wilt Virus, Tomato Yellow leaf Curl Virus, Fusarium oxysporum (Fusarium wilt), Fusarium oxysporumradicis* are given.

Table 1. Primes used to determine the resistance levels of genotypes to the diseases mentioned.

Disease Name	Marker Name	Gen*	Primer Forward	Primer Reverse
Meloidogyne spp	CAPS	MI-REX	TCGGAGCCTTGGTCTGAATT	GCCAGAGATGATTCGTGAGA
	SCAR	MI23	TGG AAA AAT GTT GAA TTT CTTTTG	GCA TAC TAT ATG GCT TGT TTA CCC
Tomato Yellow Leaf Curling Virus (Tylcv)	CAPS	TY-1	GGTACTCCTGGAAGGGTTAAGG	CACGCTGGTTCTGTTGTATCTC
	SCAR	TY-3	GGTAGTGGAAATGATGCTGCTC	GCTCTGCCTATTGTCCCATATATAACC
	SCAR	TY-2	ACCCCAAAAACATTTCTGAAATCCT	TGGCTATTTTGTGAAAATTCTCACT
Tomato Spotted Wilt Virus	SCAR	Sw-5–2	AATTAGGTTCTTGAAGCCCATCT	TTCCGCATCAGCCAATAGTGT
Fusarium oxy. radicis	SCAR	Frl	CACATTCATCATCTGTTTTTAGTCTATTC	CACAATCGTTGGCCATTGAATGAAGAAC
Fusarium Wilt	CAPS	I-2	GGGCTCCTAATCCGTGCTTCA	GGTGGAGGATCGGGTTTGTTTC
	SCAR	I3	TTCCCTCAATCCAACAAAAGTT	ACTCTCGAGTTCCGGTGAAA
Verticillium Wilt	CAPS	V2LeO3	CAAACATAGCTGGAAGAATC	TAGGAGGAAAAGAATTGG

[1]Marker amplicon sizes are as follows: MI-REX, Resistant: TaqI digested bands of 570 and 160 bp, Susceptible: remains uncleaned (750 bp); MI23, Resistant: 380 bp, Susceptible: 420 bp; TY-1, Resistant: TaqI digested bands of 500, 300 and 160 bp, Susceptible: TaqI digested bands of 500, 300 and 200 bp; TY-2, Resistant: 120 bp, Susceptible: 213 bp; TY-3, Resistant: 630 bp, Susceptible: 320 bp; Sw-5-2, Resistant: 574 bp, Susceptible: 464 bp; Frl, Resistant: 950 bp, Susceptible: 1000 bp; I-2, Resistant: FokI digested fragments of 390 and 410 bp, Susceptible: remains uncleaned (800 bp); I3, Resistant: 673 bp, Susceptible: 480 bp; V2LeO3, Resistant: HincII digested fragments of 428 and 601 bp, Susceptible: remains uncleaned (1029 bp) Genetic distances of the markers with the disease resistance traits: TY-1: 0.2 cM, TY-3: 1.4 cM, Frl: 0.016 cM, I-2: 0.1cM. Markers located inside the genes of interest: MI-REX, MI23, TY-2, Sw-5–2, I3 and V2LeO3.

Table 2. Disease and pest resistance status of *Meloidogyne incognita, Verticillium dahlia, Tomato Spotted Wilt Virus, Tomato Yellow leaf Curl Virus, Fusarium oxysporum (Fusarium wilt), Fusarium oxysporum radicis*

Genotype	*Meloidogyne incognita*resistance status (MI-REX & MI23)*	Tomato Yellow leaf Curl Virus (Ty-1/Ty-3 or Ty-2)*	Tomato Spotted Wilt Virus resistance status (Sw 5-2)*	*Fusarium* oxy. *radicis* resistance status (Frl) *	Verticillium Wilt resistance status (V2LeO3)*	Fusarium Wilt resistance status (I-2 & I-3)*
C1-1	RR	RR	RR	RR	Rr	Rr
C1-2	RR	RR	RR	Rr	RR	Rr
C1-3	RR	RR	RR	RR	RR	RR
C1-4	RR	RR	Rr	RR	Rr	RR
C1-5	RR	RR	RR	RR	rr	RR
C1-7	RR	RR	RR	Rr	RR	RR
C1-8	RR	Rr	Rr	Rr	RR	RR
C1-9	RR	RR	RR	Rr	RR	RR
C1-10	RR	RR	Rr	Rr	RR	RR
C1-11	RR	RR	RR	Rr	RR	RR
C1-12	RR	RR	Rr	RR	RR	RR
C1-13	RR	RR	RR	RR	RR	RR
C1-14	Rr	rr	rr	RR	RR	RR
C1-15	RR	rr	rr	RR	Rr	Rr
C1-16	RR	RR	Rr	RR	Rr	Rr
C1-17	RR	RR	RR	RR	Rr	Rr
C1-18	RR	RR	RR	RR	rr	rr
C1-19	Rr	rr	rr	rr	rr	rr
C1-20	RR	Rr	Rr	RR	rr	rr
C1-21	RR	RR	RR	RR	RR	RR
C1-22	RR	Rr	RR	RR	RR	RR
C1-23	RR	RR	RR	RR	RR	RR
C1-24	RR	RR	RR	RR	RR	RR
C1-25	RR	RR	RR	RR	RR	RR
C1-26	RR	rr	Rr	Rr	Rr	Rr
C1-27	Rr	RR	RR	RR	Rr	Rr
C1-28	RR	RR	RR	RR	Rr	Rr
C1-30	RR	Rr	Rr	Rr	Rr	Rr
C1-31	RR	RR	RR	RR	Rr	RR
C1-32	RR	RR	RR	RR	Rr	RR
C1-33	Rr	Rr	RR	RR	Rr	RR
C1-34	RR	RR	RR	RR	RR	RR
C1-35	RR	RR	RR	RR	RR	RR
C1-36	RR	RR	RR	RR	RR	RR
C1-37	RR	RR	RR	RR	RR	RR
C1-38	Rr	RR	RR	RR	RR	RR
C1-39	RR	RR	RR	RR	RR	RR

Table 2. Continue

Genotype	*Meloidogyne incognita*resistance status (MI-REX & MI23)*	Tomato Yellow leaf Curl Virus (Ty-1/Ty-3 or Ty-2)*	Tomato Spotted Wilt Virus resistance status (Sw 5-2)*	*Fusarium* oxy. *radicis* resistance status (Frl) *	Verticillium Wilt resistance status (V2LeO3)*	Fusarium Wilt resistance status (I-2 & I-3)*
C1-40	RR	RR	RR	RR	RR	RR
C1-41	Rr	RR	RR	RR	RR	RR
C1-42	RR	RR	RR	RR	RR	RR
C1-43	RR	RR	RR	RR	RR	RR
C1-44	RR	Rr	RR	rr	rr	rr
C1-45	rr	rr	Rr	Rr	rr	Rr
C1-46	RR	RR	RR	RR	RR	RR
C1-47	RR	RR	RR	rr	rr	rr
C1-48	RR	RR	RR	RR	RR	RR
C1-49	rr	Rr	RR	RR	RR	RR
C1-50	RR	Rr	RR	RR	RR	RR
C1-51	RR	Rr	Rr	Rr	Rr	Rr
C1-52	RR	Rr	RR	rr	rr	rr
C1-54	RR	Rr	RR	RR	RR	RR
C1-55	Rr	Rr	Rr	RR	RR	RR
C1-56	RR	RR	RR	RR	RR	RR
C1-57	RR	RR	RR	RR	RR	RR
C1-59	RR	RR	RR	RR	RR	RR
C1-60	Rr	RR	RR	RR	RR	RR
C1-61	RR	RR	Rr	Rr	Rr	Rr
C1-62	RR	Rr	RR	RR	RR	RR
C1-63	Rr	RR	RR	RR	RR	RR
C1-64	RR	rr	rr	rr	rr	rr
C1-66	RR	RR	RR	RR	RR	RR
C1-67	RR	RR	RR	RR	RR	RR
C1-68	RR	RR	RR	RR	rr	RR
C1-69	RR	RR	Rr	Rr	Rr	Rr
C1-70	RR	Rr	RR	RR	RR	RR
C1-72	RR	Rr	RR	RR	RR	RR
C1-73	RR	Rr	rr	RR	RR	RR
C1-74	RR	Rr	RR	RR	RR	RR
C1-90	RR	Rr	RR	RR	RR	rr
C1-160-4	Rr	RR	RR	rr	RR	RR

4. Discussion

Lizardo et al.(2022) reported that 8 cultivars gave a sensitive band at 430 bp as a result of the use of the Mi23 molecular marker against *M. incognita*, and that these cultivars have no resistance to *M. incognita* and the sources of resistance need to be determined. Bozbuga et al. (2020) In a study examining the levels of resistance against *Meloidogyne incognita*, it was reported that three genotypes were resistant and 96 genotypes were susceptible. Kabas et al. (2021), it was reported that 7 genotypes were heterozygous resistant (464-575 bp and 510-575), 23 were homozygous resistant (575 bp) and 10 genotypes were susceptible (464 bp) as a result of molecular investigations against TSWV. Erkan (2020b) reported that, as a result of molecular investigations against TYLCV in commercial tomato varieties, 8 varieties of TYLCV gave a sensitive band at 269 bp, and two resistant bands of 519 bp and 269 bp in 12 varieties. Basım et al.(2022) in his study examining the levels of resistance against *Tomato spotted wilt virus, Tomato yellow leaf curl virus, Meloidogyne incognita*; 34 susceptible to TYLCV, 56 heterozygous resistant, 4 homozygous resistant, 57 susceptible to T SWV, 27 heterozygous resistant, 4 homozygous resistant and 2 genotypes in which Sw5-2 marker did not work, against *Meloidogyne spp*. As a result of the use of Mi23 molecular marker, 44 sensitive, 35 homozygous resistant and 11 heterozygous resistant were determined. Mutlu et al. (2015) designed a marker for the Frl gene. Homozygous resistant at 950 bp, susceptible at 1000 bp, and heterozygous resistant individuals have been reported to give bands at 950-1000. While 14 homozygous resistant, 80 heterozygous resistant and 63 susceptible genotypes were detected against *Fusarium oxysporum* in *S. lycopersicum* genotypes, 47 homozygous resistant, 66 heterozygous resistant and 45 sensitive genotypes were determined against *Verticillium wilt* (Aydın and Aktaş 2022).

5. Conclusions

The presence of genotypes with resistance genes against diseases and pests, which cause significant problems in tomato cultivation, which has a large production area and production amount in the world, is a valuable situation in terms of breeding. As a result of the study, more than one genotype with resistance gene against the aforementioned diseases and pests was determined. C1-3, C1-13, C1-21, C1-23, C1-24, C1-25, C1-34, C1-35, C1-36, C1-37, C1-39, C1-40, C1-42, C1-43, C1-46, C1-48, C1-56, C1-57, C1-59, C1-66, C1-67 genotypes which showed homozygous resistance against all the diseases and pests mentioned in our study are the most important output of the study. At the same time, 54 of the genotypes are homozygous resistant to 5 factors (RR), heterozygous to another factor (Rr), 64 of the genotypes are homozygous resistant to 4 different disease factors (RR), heterozygous resistance to the other two factors (Rr), gene determined to be carried. Considering these data obtained, a valuable gene pool with resistance to disease factors has been created for future breeding studies.

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