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RESEARCH PAPER

Determination of The Response of Wild and Cultivated Tomato Genotypes to Some Disease and Pests by Molecular Markers

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Abstract

Fusarium oxysporum f. sp. *lycopersici, Verticillium* spp., and nematodes, as well as virus diseases that negatively affect production with limited chemical control cause significant losses in greenhouse tomato cultivation. The practical and effective side of controlling diseases is genetic control by breeding. Using a genomic approach for plant breeding is a more sustainable and effective way to control disease and pests. The development of the resistant line is improved by conventional breeding methods that can be conducted over a long period. However, molecular markers make these processes considerably shorter with identifying resistant individuals. In this study, 14 wild and 188 cultivated tomato genotypes have been tested against *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), *Meloidogyne* spp. (root-knot nematodes), Tomato spotted wilt virus (TSWV), Tomato mosaic virus (ToMV), Tomato yellow leaf curl virus (TYLCV) and *Verticillum dahliae* Kleb. (Ve) diseases and pests with using MAS (Marker- Assisted Selection) technique. According to these results, it has been determined that the selected markers can be used effectively in breeding studies to determine the diseases mentioned above.

1. Introduction

The cultivated tomato (*Solanum lycopersicum* L.) is a self-pollinated plant with 2n=24 chromosomes and its origin is Middle and South America. It is thought that it has been brought to the European continent in the 16th century from Peru, Ecuador and Bolivia. It is a very popular type of vegetable that entered Turkey in the 1900s (Kaya et al., 2018). It has a genetic potential since it has been cultivated in the world from ancient times to the present day. It is very important to determine these genetic differences and using inbreeding studies based on biotic and abiotic stress that may occur today and in the future. There are serious crop losses due to these diseases and pests during cultivation. Therefore, disease and pest resistance genotypes are very important in tomato breeding studies. On the other hand, intensive use of pesticides for plant pests and diseases both negatively affect the environment and human health. For this reason, the search for less chemical control or alternative methods continues in Turkey and the world. The most important way is to develop resistant varieties against diseases and pests. The common fungal diseases encountered in greenhouse tomato cultivation in Turkey are *Fusarium oxysporum* f. sp. *lycopersici* and *Verticillium* spp. These soil-borne diseases cause significant economic damage. In addition, it is reported that yield losses caused by virus diseases negatively affect and have no chance of chemical control (Hanson et al., 2016). For this reason, studies on developing resistant cultivars are very important in the control of viruses. For resistance studies, it is necessary to identify the virus effectively, to know its molecular structure and disease mechanism. Various methods have been

used in the identification of viral diseases. Multiple identification methods such as biological indexing, cellular structures, vector transport status, serological tests, particle morphology, and molecular methods can be used to characterize any virus in plant diseases. World tomato cultivation is restricted because of many virus diseases which are not controlled by chemicals. The most common of these viruses are; Tomato Mosaic Virus (ToMV), Tomato yellow leaf curl virus (TYLCV), Cucumber mosaic virus (CMV), Tomato spotted wilt virus (TSVW), Potato Y Virus (PVY), Tomato chlorosis virus (ToCV), Tomato brown rugose fruit virus (ToBRFV), which has been increasing rapidly of last years, can cause significant damage in Turkey and the world (Guldur et al.,1994; Yurtmen et al.,1999; Nie and Singh, 2002; Mason et al., 2003; Çevik and Erkış, 2007; Fidan, 2020). Molecular markers have been used extensively in many plants since 1980 in the development of breeding lines that are resistant or tolerant to these viruses. In tomatoes, the use of molecular marker techniques in disease resistance has been developed in the last 10 years and marker-assisted selection (MAS) is a quite common method that has been used in plant breeding programs by researchers (Grube et al., 2000). Owing to these markers, screening of the genotypes to biotic factors and use for breeding programs is more rapid and reliable. Inbreeding studies, MAS selection can be used against the *Fusarium*, *Verticillium,* bacterial spot disease, ToMV, TYLCV and Root-Knot Nematode routinely.

The advantages of MAS studies can be summarized as follows:

- It is an easier method than phenotypic screening.
- It saves time by providing selection during the germination phase.
- Its reliability is high as it is not affected by environmental factors.
- Allows the selection of a single plant by selecting homozygous and heterozygous plant.
- Contributes to the creation of breeding records by determining the genetic status
- It provides a more precise and accurate selection of genotypes with specific characteristics.
- In recent years, markers such as Randomly Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), Sequence Characterized Amplified Region (SCAR), Cleaved Amplified Polymorphic Sequence (CAPS), Single Nucleotide Polymorphism (SNP) and Insertion-Deletion (InDel) have been used in tomato. It is one of the common techniques used to identify genotypes (Gebhardt, 2007).

In this study, tomato lines, other characteristics (yield, fruit quality, plant height, fruit weight, plant vigor etc.), determined according to IPGRI (International Plant Genetic Research Institute) rules, were used. The purpose of the study was to test these lines against some diseases and pests such as *F. oxysporum* f. sp. *radicis-lycopersici*, *V. dahliae*, Tomato spotted wilt virus, Tomato mosaic virus, Tomato yellow leaf curl virus, *Meloidogyne* spp., by using molecular marker methods and to provide material for future tomato breeding programs.

2. Materials and Methods

A total of 202 tomato genotypes including released or commercial cultivars up to F5-F8 level for breeding program and accessions were used in this research. Tomato accessions (LA series) were provided by the Tomato Genetics Resource Center, University of California, Davis, CA 95616 (TGRC, 2014).

All tomato genotypes were used for testing against *F. oxysporum* f.sp. *radicis-lycopersici*, *Meloidogyne* spp., Tomato spotted wilt virus, Tomato mosaic virus, Tomato yellow leaf curl virus and *V. dahliae*. LA 2830 (*S. habrochaites*), LA 0722 (*S. pimpinellifolium*), LA 4442 (*S. penelli*), LA 4353 (*S. lycopersicum* var. *cerasiforme*), LA 4252 (*S. pimpinellifolium*), LA 3325 (*S. juglandifolium*), LA 2332 (*S. habrochaites*). For DNA extraction, 20 seeds of each line were sown in seeding dishes filled with substrates that were artificially mixed with a proportion of peat: perlite in a 1:1 ratio. When the plants had 3-4 true leaves, they were used for DNA analysis.

DNA isolation was performed according to the CTAB method developed by Doyle and Doyle (1987). In this method, 0.1 g of young leaf samples taken from each genotype were thoroughly crushed in a mortar with liquid nitrogen (-195°C), then the leaf frozen transferred into 2.2 ml microfuge tubes and added 0.9 ml extraction buffer [(2% w/v) CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris–HCl (pH 8.0), 1% PVP, 0.1% sodium bisulfite, and 0.2% (v/v) 2-mercaptoethanol] tubes to which buffer solution was added were kept in a hot water bath at 65°C for 1 hour with stirring every 10 minutes. The samples removed from the water bath were kept at room temperature for 5-10 minutes, and then 0.9 ml of chloroform: isoamyl alcohol (24:1) was added into the tubes. Tubes containing chloroform-isoamyl alcohol were mixed continuously and very slowly for 15 minutes and then centrifuged at 14000 rpm for 15 minutes. The supernatants of the centrifuged samples were taken (approximately 0.75 ml) and transferred to a new tube, and cold (incubated at-20°C) isopropanol (0.5 ml) was added to it, and the tubes were shaken slowly and the DNA was allowed to precipitate. The supernatant was then removed from the tube. Then, 1 ml of 76% ethanol containing 10 mM ammonium acetate was added to the tube and the tubes were shaken for 15 minutes. After the washed DNA was centrifuged at 2000 rpm for 30 seconds, the supernatant was poured and the DNA was dried at room temperature. The dried DNA was thawed after one day by adding ultrapure water.

Then, the concentration of the obtained DNA was adjusted to 20 ng μl^{-1} for use in PCR analysis.

The DNA markers used in the research are given in Table 1. PCR conditions vary for each DNA marker, but in each reaction solution 1X PCR buffer (50 mM KCl, 10 mM Tris-HCL, pH 9.0, 0.1% Triton X-100), 0.2 mM dNTPs, 2 mM $MgCl_2$, 0.5 U Taq Polymerase, 5 pmol forward and reverse primer and 20-100 ng DNA sample were added. In the reactions, the first denaturation was started at 94°C for 5 minutes and the cycle was performed 37 times, including denaturation at 94°C for 30 seconds, annealing at 59°C for 30 seconds (it may vary according to each DNA marker) and 45 seconds at 72°C, and this cycle was performed for 5 minutes at 72°C. The PCR products obtained as a result of the study were conditioned on 2-3% agarose gel and the results were evaluated. When the 2-3% agarose gel used was insufficient, high-resolution agarose gel was used in the study. The gel was visualized in a UV device after staining with 0.5 mg ml^{-1} ethidium bromide solution.

3. Results and Discussion

In this study, 14 wild tomatoes genotype and 188 *S. lycopersicum* genotypes were screened with 6 SCAR and 1 CAPS marker providing resistance against nematode (Mi), *V. dahliae*, *F. oxysporum* f. sp. *radicis-lycopersici*, Tomato spotted wilt virus, Tomato yellow leaf curl virus and Tomato mosaic virus.

Pmi markers developed in association with the Mi-1.2 gene were used for resistance to nematodes. Homozygous resistance band of 550 bp and susceptible genotype was determined in 350 pb with Pmi3 marker (Table 2). According to these results, 32 homozygous resistant, 1 heterozygous resistant and 139 susceptible genotypes were determined in the cultivated tomato (*S. lycopresicum*) genotype (Table 3). While no resistance was found in S. pennelii (LA4442), S. peruvianum, *S. lycopersicum* var. *cerasiforme* (LA4353), *S. juglandifolium* (LA3325) and *S. pimpinellifolium* (LA4252, LA0722), 1 in *S. habrochaites* (LA0407) line with resistance to nematodes was determined. Devran and Elekçioğlu (2004), tested the Pmi and Mi23 SCAR markers and found similarly homozygous-susceptible at 350 bp homozygous-resistance bands at 550 bp in tomato lines. Generally, studies show that *S. peruvianum* species have nematode resistance (El Mehrach et al., 2005; Seah et al., 2007; Kaur et al., 2014). However, according to the results of our research, nematode resistance was not found in the *S. peruvianum* line. However, genotypes with nematode resistance were determined in *S. lycopersicum* and *S. habrochaites* species. In this case, it is thought that the origins of the cultivated tomato (*S. lycopersicum*) may have crossed with different resistance wild types lines.

Ve2 marker, developed in association with the Ve gene, was used for resistance to Verticillium wilt and 242 bp of resistance and 131 bp of susceptible bands were obtained. According to these results, 47 homozygous-resistant, 66 heterozygous-resistant and 45 susceptible genotypes were determined in the *S. lycopresicum* genotype (Table 3). *S. pennelii* (LA4442) and *S. peruvianum* genotype had one

Table 1. Primer sequences used in the study.

Gene	Marker	Primer sequence		Marker type	References
Tm-2a	Tm2	F: CAC CTT TCC CTC TCC AA R: CAC CTT TCC CCT AAA GC	Co-dominant	SCAR	Dax et al. (1998)
Ve	Ve2	F:GGA TCT TAG CTC ACT TTA TGT TTT GAA C R: GGT GCT GGT TTC AAC TCT GAA GT	Co-dominant	SCAR	Kawchuk et al. (2001)
Mi	PMi3	F:GGT ATG AGC ATG CTT AAT CAG AGC TCT C R:CCT ACA AGA AAT TAT TGT GCG TGT GAA TG	Co-dominant	SCAR	El Mehrach et al. (2005)
Sw-5	Sw5-2	F:AAT TAG GTT CTT GAA GCC CAT CT R:TTC CGC ATC AGC CAA TAG TGT	Co-dominant	SCAR	Dianese et al. (2010)
Ty3	P6-25	F:GTA GTG GAA ATG ATG CTG CTC R:CTC TGC CTA TTG TCC CAT ATA TAA CC	Co-dominant	SCAR	Ji et al. (2007a)
Ty1	JB1	F: AAC CAT TAT CCG GTT CAC TC R: TTT CCA TTC CTT GTT TCT CTG	Co-dominant	CAPS	De Castro et. al. (2007)
Frl	$SCAR_{Frl}$	F:TTG GCC ATT GAA TGA AGA AC R: CAT CTG TTT TTA GTC TAT TC	Co-dominant	SCAR	Mutlu et al. (2015)

Table 2. DNA fragment sizes (bp) identify resistant and susceptible individuals linked to resistance genes.

Resistance	Tm-2a	Ve	Mi	Sw	Ty3	Ty1	Frl
			Base pairs (bp)				
H	703	242	550	574	630	500	1000
R	703-538	242-131	550-350	574-470	630-350	500-400	1000-950
S	538	131	350	510-470	350	400	950

H: Homozygous resistant, R: Heterozygous resistant, S: Susceptible

Table 3. Tomato genotypes used in the study and their resistance situation (number).

	Tm-2a	*Ve*	*Mi*	*Sw*	*Ty3*	*Ty1*	*Frl*
S. lycopersicum							
H	16	47	32	6	22	17	14
R	11	66	1	7	4	1	80
S	85	45	139	121	128	153	63
S.pimpinellifolium							
H	0	2	0	0	0	0	0
R	1	2	0	0	0	0	2
S	2	0	4	2	4	4	1
S.penelli							
H	0	0	0	0	0	0	0
R	0	1	0	0	0	0	0
S	2	0	2	2	2	2	2
S.habrochaites							
H	1	3	1	0	0	0	1
R	1	1	0	0	0	0	1
S	2	0	4	5	5	5	3
S.peruvianum							
H	0	0	0	0	0	0	0
R	0	1	0	0	0	0	1
S	1	0	1	1	1	1	0
S.lycopersicum var. *cerasiforme*							
H	0	1	0	0	0	0	0
R	0	0	0	1	0	0	1
S	0	0	0	0	0	0	0
S. juglandifolium							
H	0	1	0	0	0	0	0
R	0	0	0	0	0	0	0
S	1	0	1	1	1	1	1

H: Homozygous resistant, R: Heterozygous resistant, S: Susceptible

heterozygous resistance, one homozygous resistance in *S. lycopersicum* var. *cerasiforme* (LA4353) and *S. juglandifolium* (LA3325) genotypes, two homozygous resistance in *S.pimpinellifolium* (LA0722 and LA4252) species two heterozygous resistance, three homozygous and one heterozygous resistance against Verticillium wilt were determined in *S. habrochaites* (LA 2830 and LA2332) line (Table 3). In different studies, it has been reported that Ve1 and Ve2 markers developed against Verticillium wilt were resistant in the range of 242 bp and susceptible in the range of 131 bp and could be easily used in MAS studies (Acciaari et al., 2007; Morid et al., 2017).

SCARFrl marker was used for resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL). Accordingly, resistance bands were obtained in 1000 bp and susceptible bands in 950 bp. 14 homozygous resistant, 80 heterozygous resistant and 63 susceptible genotypes were determined in *S. lycopersicum* (Table 3). Two susceptible genotypes in *S. pennelii* (LA4442) species, one heterozygous resistant in S. peruvianum and *S. lycopersicum* var. *cerasiforme* (LA4353) species, one susceptible in *S. juglandifolium* (LA3325) and two heterozygous resistants, one susceptible in *S. pimpinellifolium* (LA0722) species, *S. habrochaites* (LA 2830, LA2332) one homozygous, two heterozygous resistant and three susceptible genotypes were determined (Table 3). It has been determined that there are many genotypes of FORL resistance used in our research. For this reason, is thought to be the genetic resistance of FORL controlled by a single dominant gene (Roberts et al., 2000). In addition, in a study, *S. peruvianum* is shown as a source of FORL resistance (Laterrot and Moretti, 1991). It has been reported that the SCARFrl marker (1000 bp resistant and 950 bp susceptible) that we used in our research was easily used in other studies and very successful results were obtained (Mutlu et al., 2015). These studies support our study (Table 3).

It was tested with the Sw-5-2 SCAR marker, which was developed in association with the Sw-5 gene, which provides resistance to Tomato spotted

wilt virus. Resistant bands were obtained with the relevant marker at 574 bp and susceptible bands at 470 bp. In *S. lycopresicum* genotypes, 6 homozygous, 7 heterozygous resistant and 121 susceptible genotypes were determined (Table 3). One susceptible in *S. peruvianum* and *S. juglandifolium* (LA3325) species, two susceptible in *S. pimpinellifolium* (LA0722) and *S. pennelii* (LA4442) species, 5 susceptible genotypes in S. habrochaites (LA2332, LA2830) and *S. lycopersicum* var. *cerasiforme* (LA4353) 1 heterozygous resistance was determined (Table 3). Nascimento et al. (2009), in their study with tomato genotypes, screened populations created by hybridization of elite lines known to be resistant to tomato spotted wilt virus with the SCAR marker developed for resistance and reported that the marker was useful in distinguishing heterozygous and homozygous lines. Again, in a similar study conducted by Fidan and Sarı (2019) on tomatoes, the band range of the Sw-5 gene are similar to our study.

In the test for resistance to tomato yellow leaf curl virus, 2 markers (JB1 for Ty-1 and P6-25 for Ty-3) developed with Ty1 and Ty3 alleles, which are 2 of the 4 genes that provide the marker resistance, were used. For Ty1, homozygous resistance was obtained with the JB-1 CAPS marker at 500 bp, while susceptible bands were obtained at 400 bp (De Castro et al., 2007). For Ty3, homozygous resistance was obtained at 630 bp with the P6-25 SCAR marker, while susceptible bands were obtained at 350 bp (Ji et al., 2007a). According to results, in *S. lycopresicum* genotypes, 17 homozygous, 1 heterozygous resistant and 153 susceptible genotypes were determined for the Ty1 gene (Table 3). One each in *S. peruvianum* and *S. juglandifolium* (LA3325), 4 susceptible in *S. pimpinellifolium* (LA0722, LA4252), 2 susceptible in *S. pennelii* (LA4442) species, 5 susceptible in *S. habrochaites* (LA2332, LA2830) species and no result was obtained for *S. lycopersicum* var. *cerasiforme* species (Table 3).

In *S. lycopresicum* genotypes, 22 homozygous, 4 heterozygous resistant and 128 susceptible genotypes were determined for the Ty3 gene (Table 3). One each in *S. peruvianum* and *S. juglandifolium* (LA3325), 4 susceptible in *S. pimpinellifolium* (LA0722, LA4252), 2 susceptible in *S. pennelii* (LA4442), 5 susceptible in *S. habrochaites* (LA2332, LA2830) and *S. lycopersicum* var. *cerasiforme* species, no results were obtained.

It has been demonstrated in all of the studies carried out to date that Ty1 and Ty3 with the widest spectrum among the 6 genes (Ty1, Ty2, Ty3, Ty4, Ty5, Ty6) detected in the development of lines resistant to TYLCV are effective sources of resistance. Ty-1 and Ty-3 have been adopted by different researchers as the markers that show resistance to TYLCV virus in tomatoes and are published in MAS (Zamir et al., 1994; Agrama and Scott, 2006; Ji and Scott, 2007a; Ji et al., 2007b; Lee et al., 2015). A study determined that *S. peruvianum* (LA1589) species showed resistance to TYLCV (Anbinder et al., 2009). However, it was determined that the *S. peruvianum* species used in our study did not show any resistance. While resistance was found in only *S. lycopresicum* species, it was determined that other species were susceptible. While TYLCV was mostly seen in genotypes of commercial origin, it was determined to be susceptible in locally collected species. In this case, it is thought that this resistance is due to the transfer to previously commercial varieties. As a matter of fact, in a study conducted by Kaya et al. (2009), in their screening with the Ty-1 CAPS marker developed for tomato yellow leaf curl virus in the tomato population in the F3 stage, 15 of 131 plants were determined to be heterozygous susceptible and no homozygous resistance could be obtained in any of the plants in a study in which wild species were predominantly used, it was determined that the lines of *S. chilense*, LA1932, LA2779 and LA1938 were resistant to TYLCV (Agrama and Scott, 2006). Again, in a similar study, it was reported that TYLCV resistance could be found in *S. pimpinellifolium*, *S. peruvianum*, *S. chilense*, and *S. habrochaites* species (Ji et al., 2007b). However, it was determined that the wild lines used in our study did not have any resistance against TYLCV virus disease. It is thought that this situation is caused by the different lines used within the species.

It was tested with the Tm2 SCAR marker, which was developed with the Tm-2a gene, which provides resistance to Tomato mosaic virus (Zengin and İlbi, 2016). With the relevant marker, 703 bp of the resistance band and 538 bp of the susceptible band were obtained. In *S. lycopresicum* genotypes, 16 homozygous, 11 heterozygous resistant and 85 susceptible genotypes were determined (Table 3). *S. peruvianum* and *S. juglandifolium* (LA3325) 1 heterozygous resistant, *S. pimpinellifolium* 1 heterozygous resistant and 2 heterozygous resistant (LA0722), *S. pennelii* (LA4442) 2 heterozygous resistant, *S. habrochaites* 1 homozygous resistant (LA2830), 1 heterozygous, 2 susceptible (LA2332) genotypes were determined and no results were obtained for *S. lycopersicum* var. *cerasiforme* (LA4353) (Table 3).

The most common genes conferring tolerance to tomato mosaic virus are known as Tm-1, Tm-2 and Tm-22 (Pelham, 1966; Hall, 1980; Levesque et al., 1990; Lanfermeijer et al., 2003; Foolad, 2007). The Tm-1 gene is also found on chromosome 5 in the *S. habrochaites* (Levesque et al., 1990; Ohmori et al., 1996; Foolad and Sharma, 2004; Foolad, 2007). Tm-2 and Tm-22 were identified on chromosome 9 in *S. peruvianum* species (Hall, 1980; Tanksley et al, 1992; Ohmori et al., 1995; Pelham, 1966; Lanfermeijer et al., 2003). However, it is known as the Tm-2 gene, which is the most widely used in the molecular marker-based selection method (Foolad and Sharma, 2004).

4. Conclusion

Among the most common fungal diseases encountered in greenhouse tomato cultivation, soil-borne diseases and pests such as *F. oxysporum* f. sp. *lycopersici, Verticillium* spp., nematodes, and virus diseases are very difficult to control. Therefore, developing cultivars that can tolerate these diseases and pests during cultivation is one of the most important strategies. It may take a long time to develop a variety with conventional breeding methods, but these periods can be shortened by molecular markers that have developed in recent years. In this study, breeding lines of which all characteristics were determined before, have been tested against *F. oxysporum* f. sp. *radicis-lycopersici*, *Meloidogyne* spp., Tomato spotted wilt virus, Tomato mosaic virus, Tomato yellow leaf curl virus and *V. dahliae* diseases. It has been determined that these markers can be used in future breeding studies in light of this information and the disease and pest resistance of some wild lines.

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