

Determination of Some Disease Resistance Genes of Tomato Breeding Lines Which Developed from Different Origine Using Molecular Markers

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Received: 22.06.2023; Accepted: 04.12.2023; Published Online: 28.02.2024

ABSTRACT

Tomato is one of the important vegetables produced in the world, and China, USA and Turkey are among the important producers in world production. Diseases and pests are one of the most important problems in tomato as in other species. Breeding for disease resistance has been an important objective in tomato improvement. When preparing new breeding programs, genetic materials from different backgrounds are needed. However, the genes for resistance to diseases and pests may vary according to the tomato production regions. In order to obtain the desired resistance in breeding programs, it is important to know which resistance is needed for which region. From this point of view, in this study, it is aimed to determine the distribution of disease and pest resistance of the lines developed from tomato varieties produced in Turkey, USA and Jordan. In the materials selected from Turkey in 2014, disease resistance other than *Verticillium* was rarely found, while Sw-5 and Ph3 genes were found in 2017 and 2019. On the other hand, it was determined that the majority of genetic materials originating from USA and Jordan had Ve, FF, Mi1-2, Pto, Tm-2, Ty-3, Ph3 and SW-5 genes. The findings show that it can be taken into account in developing disease and pest resistant varieties for different production regions.

Keywords: Tomato, Molecular markers, MAS

INTRODUCTION

Tomato is one of the most important vegetable species produced in Turkey as well as in the world, and it ranks third with a production of 10 million tons in the world production of 129 million tons/year (FAO 2021). Among the important limiting factors in tomato cultivation; They are yield are quality losses caused by biotic factors such as viruses, bacteria, nematodes and fungi. Three strategies are adopted to control the spread of diseases and pests. These are chemical treatments, cultural practices and the use of resistant varieties. Although chemical applications can prevent the spread of some diseases and pests, their usability is limited in terms of posing risks for farmers, increasing input costs and residue problems. Disease and pest control with chemical and cultural practices is not always possible. In this case, the use of resistant varieties emerges as the most economical and environmentally friendly control method.

In intensively cultivated plant species such as tomatoes, more than one disease and harmful factor can be found in production areas at the same time; it causes great losses in terms of yield and quality. Many plant species and cultivars are resistant to one or more diseases and pests. Especially hybrid varieties used in vegetable production have multiple resistances. Resistance to diseases and pests in tomato is controlled by a single gene and is dominant. Up to now, 15 resistance genes have been transferred to tomatoes cultivated from different sources (Barone 2004).

Molecular markers have been used extensively in many plants since 1980. Especially in tomato, markers related to disease resistance genes are developed and used in breeding programs. More than 40 genes (single gene and QTL) have been mapped so far. These mapped features are used in breeding programs with the marker assisted selection (MAS) method. Since 1990, the MAS method has been used in breeding programs alongside the classical breeding method. Reasons for using MAS; shortening the breeding period with early selection, facilitating the transfer of resistance by backcrossing, facilitating the breaking of the linkage between genes, facilitating the determination of traits that are difficult to screen, not needing to test for resistance to some diseases and pests

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within the scope of quarantine, therefore with fewer plants. It can be listed as providing an advantage in terms of labor and cost by enabling work.

Tomato is among the first species in which molecular markers are suggested to be used in breeding programs for indirect selection. (Tanksley 1983; Tanksley *et al.* 1992; Foolad 2007). For example, about 30 years ago, the isoenzyme marker was used in indirect selection in breeding varieties resistant to the acid phosphatase (Aps-1 locus) nematode (Medina-Filho and Stevens 1979). The MAS method has been routinely used in tomato for the determination of resistance to some diseases for the seed companies (Panthe and Foolad, 2011). For tomato, SCAR and CAPS markers have been developed by various researchers for resistance to diseases and pests such as nematode, Tomato mosaic virus, Verticillium wilt, Fusarium wilt, Tomato spotted wilt virus and Tomato yellow leafroll virus and are used extensively in breeding programs (Barone 2004).

Countries such as USA, China, India and Turkey have an important share in tomato production in the world. Apart from these countries, tomato production has accelerated in recent years. Diseases and pests are the leading factors limiting production in different climatic and soil conditions where tomato production is made. However, since every disease and pest does not have the same effect in every region, the disease and pest resistance desired by the producers differs according to the production regions. This demand may also change from year to year. Seed companies strive to develop tomato varieties with the desired strengths for each region. From this point of view, in this study, it was aimed to determine the distribution of disease and pest resistance of the lines developed from tomato varieties produced in Turkey, USA and Jordan.

MATERIALS AND METHODS

In present study United Genetics Turkey Seed Company breeding lines used as a genetic material. It was determined that the majority of genetic materials originating from USA and Jordan had Ve, I2, I3, Mi1-2, Pto, Tm-22, Ty-3, Ph2, Ph3 and Sw-5 genes (Table 1). Total 44 tomato lines were used as materials. 16 of them were from Turkey, 17 of them were USA and 11 of them were from Jordan (Table 2). Tomato seeds sown in viols containing 1:1 peat:perlite medium, after routine maintenance, and after two true leaf stages, DNA isolation from fresh leaves was made according to the CTAB method modified according to Doyle and Doyle (1990). The DNA samples obtained were run on a 1% agarose gel and the amount was determined and equalized to 20 ng in a 15µL PCR reaction. And, the markers developed for the Ve, I2, I3, Mi1-2, Pto, Tm2², Ty-3, Ph2, Ph3 and Sw-5 genes are given in Table 1. The PCR conditions specified in the relevant publications were applied and the obtained PCR product was carried out in agarose gel electrophoresis at 110 V for 3.5 hours on a 2% agarose gel. The gel was visualized on a UV imaging device (Quantum-Gel Doc. Imaging-VILBER) after staining with 0.5 mg/ml Syber safe solution. Scoring was made according to the obtained image. All these studies conducted in United Genetics Turkey Seed Company' s Molecular Biology Laboratory and greenhouses, in Bursa/Turkey.

Table 1. Molecular markers used in testing tomato lines.

Pathogen	Gene	Primer Name	Reference
<i>Verticillium dahliae</i>	<i>Ve1</i>	Ve1_SNP	Arens <i>et al.</i> (2010)
<i>F. oxysporum</i> race 1	<i>I2</i>	Z1063F/R	Arens <i>et al.</i> (2010)
<i>F. oxysporum</i> race 3	<i>I3</i>	NC-I3-017	Zhang and Panthee (2021)
<i>M. incognita</i>	<i>Mi1-2</i>	PMiF3/R3	Arens <i>et al.</i> (2010)
<i>P. syringae</i> pv. <i>tomato</i> (Pst) race 0	<i>Pto</i>	NC-Pto-001	Zhang and Panthee (2021)
Tomato mosaic virus	<i>Tm2²</i>	NC-Tm2-021	Zhang and Panthee (2021)
Tomato spotted wilt virus	<i>Sw5</i>	NC-Sw5-011	Zhang and Panthee (2021)
Tomato yellow leaf curl virus	<i>Ty-3</i>	PMiF3/R3	Arens <i>et al.</i> (2010)
Late Blight	<i>Ph-2</i>	UF-Ph2-1	Shekasteband <i>et al.</i> (2015)
Late Blight	<i>Ph-3</i>	Ph-3-GLR	Ren <i>et al.</i> (2019)

RESULTS AND DISCUSSION

In this study, resistance to Nematode, Verticillium wilt, Fusarium wilt, Tomato spotted wilt virus, Tomato yellow leaf curl virus, Bacterial speck, Late blight diseases were determined with different SCAR and CAPS markers in 44 tomato genotypes of different origins and years (Table 2).

Ve1_SNP marker developed in association with the Ve gene was used for resistance to Verticillium wilt. With Ve1_SNP marker, 476 bp of resistance and 158 bp of sensitive band were obtained and 41 resistant and 3 sensitive individuals were determined from 44 advanced tomato lines. When the lines are evaluated according to their origins, Ve resistance was observed in all the lines belonging to the Jordan region, and Ve resistance was determined in 15 lines out of 17 lines belonging to the USA region out of 16 lines belonging to the Turkey region (Table 2). Acciarri *et al.* (2007) reported that Ve1 and Ve2 markers developed for resistance to Verticillium wilt in tomato, in their study with Italian tomato genetic materials, reported that the two markers were able to distinguish the allelic difference in the Ve locus and that these markers could easily be used for MAS.

For resistance to Fusarium wilt, the Z1063 marker developed in association with the I-2 allele was used. With the Z1063 dominant SCAR marker, resistance bands from 44 lines to 25 lines were obtained in 940 bp, while no bands could be obtained in 19 of them. When the lines are evaluated according to their origins, it has been determined that the resistance is more common in lines originating from Turkey and the USA. For resistance to Fusarium wilt, the marker NC-I3-017 was tested in association with the I-3 allele. According to the linked marker, I-3 resistance allele was determined only in 4 of the 13 lines originating from the USA. I-3 strength was found on lines originating from Turkey and Jordan (Table 2). Bulbul *et al.* (2022) aimed to perform marker assisted selection (MAS) by integrating molecular markers linked to the resistance genes against bacterial, viral and fungal diseases common in tomato into the breeding programs, used this NC-I3-017 marker and they determined Fusarium wilt resistance tomato genotypes using linked marker with I-3 gene.

El Mohtar *et al.* (2007) used marker developed for the I-2 gene that provides resistance to lycopersici race 2, the expected result was obtained in 39 genotypes of 40 tomato genotypes known to be resistant to the same race, only 1 genotype had the I-3 allele, and this method was performed by molecular and classical tests conducted in three different countries. studies have been reported to validation.

In present study, PMi SCAR marker, which was developed linked to the Mi1-2 gene, which provides resistance to nematodes, was tested. Homozygous resistance band in 550 bp and homozygous susceptibility band in 350 bp were obtained for Nematode resistance. Homozygous resistance band was obtained in 17 of 44 advanced stage lines and homozygous susceptibility band in 25 of them. When the lines were evaluated according to their origins, N resistance was determined in 2 of 11 lines belonging to Jordan region, 12 lines of 16 lines belonging to Turkey region, and 11 lines of 17 lines belonging to USA region. Ty-3 resistance can also be analyzed with the same Pmi SCAR marker. With the Pmi marker, homozygous resistance band at 500 bp and homozygous susceptibility band at 350 bp are obtained for Ty-3. Homozygous resistance was detected in only 25% of 44 advanced lines tested. 9 of the Ty-3 resistant lines are from the USA region and 2 of them are from the Jordan region, and the said resistance could not be determined in the lines originating from Turkey (Table 2). Devran and Elekcioglu (2004), screened F2 plants carrying the resistance allele (Mi) to root-knot nematodes in tomato by PCR, they reported that artificial resistance tests and molecular studies support each other and that primers developed for Mi allele can be used in breeding programs.

Forty-four advanced tomato lines were tested with the NC-Sw5-011 SCAR marker, which was developed in linked with the Sw-5 gene, which provides resistance to Tomato spotted wilt virus (TSWV) in this study. With NC-Sw5-011 SCAR, resistance band was obtained at 521 bp in 23 of 44 lines and susceptibility band at 451 bp in 21 lines. It has been determined that Sw-5 resistance is very common in lines originating from the USA and widespread in lines originating from Turkey and Jordan (Table 2). The NC-Pto-001 SCAR marker, which was developed in association with the Pto gene that provides resistance to *Pseudomonas syringae*, was tested in the study. With the linked marker, homozygous resistance band was obtained in 1512 bp and homozygous susceptibility band in 450 bp. and homozygous resistance was obtained in 21 of 44 advanced lines and homozygous susceptibility bands were obtained in 23 of them. When the lines were evaluated according to their origins, Pto

resistance was determined in 5 of 11 lines belonging to Jordan region, 4 of 16 lines belonging to Turkey region, and 12 lines of 17 lines belonging to USA region.

Tomato mosaic virus (ToMV) is an extremely contagious disease in *Solanum lycopersicum*. Three genes Tm1, Tm2 and Tm2², conferring resistance to ToMV have been utilized in tomato cultivar development. Tm2² of *Solanum peruvianum* provides remarkably durable resistance against the virus. Therefore, it is used most widely in tomato breeding (Zhang *et al.* 2021). The Tm2² gene was cloned via transposon tagging (Lanfermeijer *et al.* 2003). The NC-Tm2-019 marker, which was developed related to the Tm2² gene, was used to detect ToMV resistance. With the NC-Tm2-019 co-dominant SCAR marker, resistance bands were obtained on 11 lines out of 44 lines at 885 bp, while susceptibility bands were obtained at 583 bp on 33 lines. When the lines are examined according to their origins, it was determined that the ToMV resistance was most common in the lines originating from the USA (Table2).

Late blight caused by the fungal pathogen *Phytophthora infestans* (Mont.) de Bary is one of the most destructive diseases of potato (*Solanum tuberosum*) and tomato (*S. lycopersicum*) crops under moist, cool, rainy, and humid environments (Birch and Whisson 2001; Kamoun and Smart 2005; Foolad *et al.* 2008; Shekasteband *et al.* 2015). Breeding for resistance to *P. infestans* in cultivated tomato has led to identification of three resistant genes; Ph-1, Ph-2 and Ph-3, located on chromosome 7, 10 and 9, respectively derived from *S. pimpinellifolium* (Peirce 1971; Moreau *et al.* 1998; AVDRC 1994; Shekasteband *et al.* 2015). Tomato breeders are using combinations of resistance genes to provide varieties with improved and more durable resistance (Pedersen and Leath 1988; Yang and Francis 2005; Vidavski 2008, Shekasteband *et al.* 2015). In this study UF-Ph2-1 CAPS marker used for determinate to Ph2 gene, Ph-3-GLR dominant marker used for determinate to Ph3 gene. As a result, Ph2 resistant material was not found in 44 tomato lines. For Ph3 resistance, the resistance band produced at 260 bp was determined in 29 of 44 tomato lines. It has been determined that 15 of these 29 lines are of USA origin, 7 of them are from Turkey and 7 of them are from Jordan.

Kiymacı *et al.* (2023) carried a study to determination of resistance levels to 70 tomatoes, *Meloidogyne incognita*, Tomato Yellow leaf curling virus (TYLCV), *Verticillium* wilt, *Fusarium oxysporum radices*, 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 the study. When the results of their 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 radices* (Fr1) 52 Their resistances were determined as RR (homozygous resistant), 12 Rr (heterozygous), 6 rr (sensitive).

Table 2. Molecular marker results of disease resistance of advanced tomato lines according to different origins and years.

Genotype No	Origin	Year	Disease Resistance Genes										
			<i>Ve</i>	<i>I-2</i>	<i>Mil-2</i>	<i>Pto</i>	<i>Tm-2²</i>	<i>Ty-3</i>	<i>Sw-5</i>	<i>I-3</i>	<i>Ph2</i>	<i>Ph3</i>	
S1	TURKEY	2014	RR	rr	RR	rr	RR	rr	rr	rr	rr	rr	rr
S2	TURKEY	2014	RR	rr	rr	rr	rr	rr	rr	rr	rr	rr	rr
S3	TURKEY	2014	RR	rr	rr	rr	rr	rr	rr	rr	rr	rr	rr
S4	TURKEY	2014	RR	rr	rr	rr	rr	rr	rr	rr	rr	rr	rr
S5	TURKEY	2014	RR	rr	rr	rr	rr	rr	rr	rr"	rr	rr	RR
S6	TURKEY	2015	rr	RR	RR	rr	rr	rr	rr	RR	rr	rr	RR
S7	TURKEY	2015	RR	RR	rr	rr	rr	rr	rr	rr	rr	rr	RR
S8	TURKEY	2015	RR	RR	RR	RR	rr	rr	rr	rr	rr	rr	rr
S9	TURKEY	2015	RR	RR	rr	RR	rr	rr	rr	rr	rr	rr	rr
S10	TURKEY	2015	RR	RR	rr	rr	rr	rr	rr	rr	rr	rr	rr
S11	TURKEY	2015	RR	RR	rr	rr	rr	rr	rr	rr	rr	rr	rr
S12	TURKEY	2015	RR	RR	rr	RR	RR	rr	rr	rr	rr	rr	RR
S13	USA	2016	RR	RR	rr	rr	RR	rr	RR	RR	RR	rr	RR

S14	USA	2016	RR									
S15	USA	2016	RR									
S16	USA	2016	RR									
S17	USA	2016	RR									
S18	USA	2016	RR									
S19	USA	2016	RR									
S20	USA	2016	RR									
S21	USA	2016	RR									
S22	USA	2016	RR									
S23	USA	2016	RR									
S24	USA	2016	RR									
S25	USA	2017	rr									
S26	USA	2017	rr									
S27	TURKEY	2017	RR									
S28	JORDAN	2017	RR									
S29	JORDAN	2017	RR									
S30	JORDAN	2017	RR									
S31	JORDAN	2017	RR									
S32	JORDAN	2017	RR									
S33	JORDAN	2017	RR									
S34	JORDAN	2017	RR									
S35	JORDAN	2017	RR									
S36	JORDAN	2017	RR									
S37	JORDAN	2017	RR									
S38	JORDAN	2017	RR									
S39	USA	2018	RR									
S40	USA	2018	RR									
S41	USA	2018	RR									
S42	TURKEY	2019	RR									
S43	TURKEY	2019	RR									
S44	TURKEY	2019	RR									

RR: Homozygous resistant, rr: Homozygous susceptible

CONCLUSIONS

In this study, advanced tomato lines developed from tomato varieties used in different countries and years in terms of resistance to diseases and pests that cause significant yield and quality losses in tomato production were tested with linked molecular markers. According to the results, the use of varieties resistant to different diseases has changed according to years and regions. The results show that breeding programs should be created by considering common diseases and pests, especially in important production regions, while developing tomato varieties.

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