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THE RESISTANCE OF SOME TOMATO LINES AGAINST TOMATO SPOTTED WILD VIRUS, TOMATO YELLOW LEAF CURL VIRUS AND ROOT KNOT NEMATODES (*Meloidogyne* spp.) BY MOLECULAR MARKERS

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Abstract: In this study, it was attempted to determine the resistance of different tomato lines to tomato spotted wilt virus (TSWV), tomato yellow leaf curl virus (TYLCV), and root-origin nematodes (*Meloidogyne* spp.) using molecular DNA markers. For this purpose, out of 96 different tomato lines to be tested, Sw5-2 for resistance to tomato spotted wilt ripening virus (TSWV), Ty3P6-25 for resistance to tomato yellow leaf curl virus (TYLCV) and the DNA marker Mi23, which determines resistance to root-knot nematodes (*Meloidogyne* spp.), were used by PCR. In this study, Ty3P6-25, the marker that determines resistance to TYLCV, was found to be susceptible (rr) in 34 tomato lines, heterozygous resistant (Rr) in 56 tomato lines, and homozygous resistant (RR) in 4 tomato lines. In addition, no results were obtained in 2 tomato lines. Marker Sw5-2, which determines resistance to TSWV, was found to be homozygous susceptible (rr) in 57 tomato lines, heterozygous resistant (Rr) in 27 tomato lines, and homozygous resistant (RR) in 5 tomato lines. No results were obtained in 7 tomato lines. For the marker (Mi23) that determines resistance to root-knot nematodes (*Meloidogyne* spp.), 44 tomato lines were found to be homozygous susceptible (rr), 11 tomato lines were heterozygous resistant (Rr), and 35 tomato lines were homozygous resistant. No results were obtained for 6 tomato lines. It was concluded that the DNA molecular markers used are useful in determining resistance responses to TSWV, TYLCV and *Meloidogyne* spp. in tomato and can give reproducible and reliable results in a short time.

Keywords: Root-Knot nematode, Tomato yellow leaf curl virus, Tomato spotted wilt virus, PCR, Molecular marker

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1. Introduction

Tomato, which is one of the health-promoting foods with its various minerals and vitamins, is one of the most important vegetables grown and popularly consumed all over the world. It is an annual edible fruit (*Solanum lycopersicum* L.) belonging to the *Solanum* genus of the Solanaceae family. The homeland of the tomato is South American countries such as Peru and Ecuador. The tomato was first cultivated by the Mexicans and spread from the Americas to Europe and other parts of the world after the discovery of the New World. In our country, it was cultivated in Adana in the early 1900s (Oğuz, 2010). Nowadays, it is a crop of great economic importance because of its high productivity in a short growing season and its production and consumption quantities increase every year. Our country ranks fourth after China, India and the United States with a tomato production of 13.2 million tons. Tomato production in Turkey was 10.05 million tons in total on an area of 1.79 million decares in 2010, while the production has increased by about 35% resulted with 13.2 million tons (TUIK, 2020).

In the regions where tomatoes are grown, phytopathological and entomological problems occur due to cultivation errors and the difficulties resulting from the inability to adjust the humidity, temperature and ventilation of the greenhouse conditions. Fungal and viral diseases occupy an important place in phytopathological problems. One of the most important disease factors severely affecting tomato production in Turkey as well as worldwide is viruses (Yılmaz and Sipahioğlu, 2020). The most common viral diseases of tomato growing areas are Tomato Yellow Leaf Curl Virus (TYLCV), Potato Y Virus (PVY), Tomato Mosaic Virus (ToMV), Tomato Spotted Wilt Virus (TSWV), Tomato Ring Spot Virus (ToRSV) (Wani et al., 2010), Tomato Brown Rugose Fruit Virus (TBRFV) (Salem et al., 2016; Luria et al., 2017). Root knot nematodes as plant endoparasites of the *Meloidogyne* species and cause great harm on tomato worldwide. *Meloidogyne* species include *M. incognita, M. javanica M. hapla*, and *M. enterolobii* (syn. *M. mayaguensis*) (Kiewnick

et al., 2009), but *M. incognita* is the damaging oppression species (Agrios, 2005).

Systematic breeding studies in tomato have started in 1930s. New tomato cultivars were developed in the 1950s due to increased market demands for tomatoes (Crill et al., 1971; Gardner, 1982; Gardner, 2006). With the development of molecular markers and genetic mapping methods, the opportunity to develop higher quality tomato varieties has emerged. Genetic markers and maps were firstly developed and used in tomato crop (Tanksley, 1983; Tanksley et al., 1992). Marker assisted selection (MAS) technology using molecular markers have a powerful method to solve some of the challenges associated with phenotypic selection (PS). Molecular marker is a molecular technique that can be used successfully to screen for resistance gene loci related to disease resistance in an organism. Randomly amplified polymorphic DNA (RAPD), simple sequence repeat (SSR; microsatellite), amplified fragment length polymorphism (AFLP), sequence characterized amplified region (SCAR), cleaved amplified polymorphic sequence (CAPS), single nucleotide polymorphism (SNP), and insertion-deletion (InDel) were commonly used PCR-based methods for tomato breeding purposes (Floolad and Panthee, 2012).

Genetic markers are used in both public and private sector breeding programs to determine the resistance characteristics of tomato plants against plant diseases. The MAS method is faster than phenotypic selection. It is also a cheaper and more effective method for determining some disease resistance traits. However, all known molecular markers are not sufficient for tomato breeding programs. Since most commercial tomato cultivars are developed by the private sector, they often develop their own specific molecular markers adapted to their plant germplasm pools. Studies are needed to identify allele-specific molecular markers to improve the use of MAS method in tomato breeding programs (Floolad and Panthee, 2012).

The aim of this study is to screen some tomato lines developed by our breeding program in terms of virus and nematode-resistance using DNA molecular marker specific for *TYLCV*, *TSWV* and root-not nematodes.

2. Materials and Methods

Total genomic DNA from the young leaves of 96 tomato lines (F4) from our breeding program was extracted using 3% CTAB (cetyl-triethyl-ammonium-bromide)-extraction buffer (Doyle and Doyle (1990). The DNA samples were kept at 4˚C for further use. The concentration of total genomic DNA samples from 96 tomato lines were set by a spectrophotometer (Thermo ND-1000) at 100 ng/ml. The primers used for TSWV, TYLCV and root-not nematodes are detailed in Table 1.

PCR reactions for *TSWV* and for *TYLCV* were performed in a total volume of 25 µl; of which 2.2 µl DNA, 2.5 µl 10X Dream *Taq* Buffer (containing 20 mM $MgCl_2$), 4 µl dNTP (each dNTP 2.5 mM), 0.25 µl *Taq* (5U μl^{-1} *Taq* DNA polymerase), 1 µl forward and reverse primers and nucleotide-free de-H_2O for a total volume of 25 µl.

PCR cycle parameters for *TSWV* and *TYLCV* were examined as follows: initial denaturation at 94˚C for 3 minutes, denaturation for 35 cycles at 94˚C for 30 seconds, binding for 1 minute at 53˚C, elongation for 1 minute at 72˚C, and an additional 10 minutes final extension at 72˚C.

PCR reaction parameters of the PCR for *Mi*23: 3 minutes of initial denaturation at 94˚C, 35 cycles of denaturation at 94˚C for 30 seconds, binding for 1 minute at 57˚C, 1 minute of elongation at 72˚C, and an additional 10-minute final elongation at 72˚C.

The PCR products were separated by gel electrophoresis in 1.5% agarose gel with 0.5 TAE (Tris-acetate-EDTA) buffer. The agarose gel was stained in the ethidium bromide (0.5 mg/ml) for 10 minutes. The PCR results were visualized and recorded by using an ultraviolet (UV) light imaging system (Vilber Lourmat, France).

Table 1. Molecular marker and primer sequences used in study

Gene	Marker	Primer Sequences 5'……3'	References
Sw5	Sw5-2	F:AATTAGGTTCTTGAAGCCCATCT R:TTCCGCATCAGCCAATAGTGT	Dianese et al.,2010
Ty	Ty3 P6-25	F:GGTAGTGGAAATGATGCTGCTC R:GCTCTGCCTATTGTCCCATATATAACC	Jensen et al.,2007
Mi	Mi23	F:TGGAAAAATGTTGAATTTCTTTTG R:GCATACTATATGGCTTGTTTACCC	Seah et al., 2007

3. Results

The result of PCR studies with primers *Ty3*P6-25, *Sw5*-2 and *Mi*23: For *Ty3*P6-25, homozygous resistant (*RR*) genotypes produced a single band of 630 bp, while heterozygous (*Rr*) had two bands of 630 and 320 bp, and susceptible genotypes (*rr*) were found to have a single band of 320 bp (Table 2, Figure 1).

In *Sw*5-2, homozygous resistant (*RR*) genotypes showed a single band of 574 bp, while heterozygous (*Rr*) showed two bands of 574 and 470 bp and a single band of 470 bp was found in susceptible genotypes (*rr*) (Figure 2, Table 2).

On the other hand, in *Mi*23 gene, homozygous resistant (*RR*) genotypes formed a single band with a length of 380 bp, while in heterozygous genotypes (*Rr*) it was displayed as two bands of 380 and 430 bp, while in susceptible genotypes (*rr*) a single band of 430 bp was found (Figure 3, Table 2).

Table 2. Genotypic features of tomato lines analysed by PCR

Tomato Line #	*Ty3*P6	*Sw5*-2	*Mi*23	Tomato Line #	*Ty3*P6	*Sw5*-2	*Mi*23
1	*rr*	-	*rr*	49	*Rr*	*rr*	*rr*
2	-	-	*rr*	50	*Rr*	*Rr*	*rr*
3	*rr*	*rr*	*RR*	51	*Rr*	*rr*	*RR*
4	*rr*	*rr*	*RR*	52	*Rr*	*rr*	*RR*
5	*Rr*	*rr*	*rr*	53	*rr*	-	*rr*
6	*Rr*	*rr*	*rr*	54	*rr*	*rr*	*rr*
7	*Rr*	*rr*	*RR*	55	*Rr*	*rr*	*RR*
8	*Rr*	*rr*	*RR*	56	*Rr*	*rr*	*RR*
9	*Rr*	*Rr*	-	57	*Rr*	*rr*	*rr*
10	*Rr*	*Rr*	*rr*	58	*Rr*	*Rr*	*rr*
11	*Rr*	*Rr*	*rr*	59	*Rr*	*rr*	*RR*
12	*Rr*	*rr*	*rr*	60	*rr*	*Rr*	*RR*
13	*rr*	*rr*	*RR*	61	*rr*	*Rr*	*rr*
14	*rr*	*rr*	*RR*	62	*rr*	*Rr*	*rr*
15	*RR*	*Rr*	*Rr*	63	*Rr*	*Rr*	*rr*
16	*RR*	*rr*	-	64	*Rr*	*RR*	*rr*
17	*rr*	*rr*	*RR*	65	*Rr*	*Rr*	*rr*
18	*Rr*	*rr*	-	66	*Rr*	*rr*	*rr*
19	*rr*	*Rr*	*RR*	67	*rr*	*rr*	*RR*
20	*Rr*	*rr*	*Rr*	68	*rr*	*rr*	*RR*
21	*RR*	*Rr*	*rr*	69	*Rr*	*rr*	*RR*
22	*rr*	*Rr*	*rr*	70	*Rr*	*rr*	*RR*
23	*rr*	*Rr*	*RR*	71	*Rr*	*Rr*	*RR*
24	*rr*	*rr*	*RR*	72	*Rr*	*rr*	*Rr*
25	*Rr*	*Rr*	*rr*	73	*Rr*	*rr*	*RR*
26	*rr*	*Rr*	*rr*	74	*Rr*	*rr*	*RR*
27	*rr*	*rr*	*rr*	75	*Rr*	*rr*	*rr*
28	*Rr*	*rr*	*rr*	76	*rr*	*Rr*	*rr*
29	*Rr*	*rr*	*rr*	77	*rr*	*Rr*	*Rr*
30	*Rr*	*rr*	*rr*	78	*Rr*	*rr*	*Rr*
31	*Rr*	*rr*	*rr*	79	*Rr*	*Rr*	*rr*
32	*Rr*	*rr*	*rr*	80	*Rr*	*rr*	*rr*
33	*Rr*	*rr*	-	81	*rr*	*rr*	*Rr*
34	*Rr*	*rr*	*rr*	82	*Rr*	*rr*	*Rr*
35	*Rr*	*rr*	-	83	*rr*	*rr*	*Rr*
36	*Rr*	*rr*	*RR*	84	*rr*	*rr*	*Rr*
37	*rr*	*Rr*	*RR*	85	*rr*	*RR*	*rr*
38	*rr*	*rr*	*RR*	86	*Rr*	*Rr*	*rr*
39	*rr*	*rr*	*RR*	87	*rr*	*Rr*	*rr*
40	*rr*	*Rr*	*RR*	88	*Rr*	*Rr*	*rr*
41	*rr*	*rr*	-	89	*Rr*	*rr*	*RR*
42	*rr*	*rr*	*RR*	90	*Rr*	*RR*	*RR*
43	*rr*	*rr*	*RR*	91	*Rr*	*RR*	*Rr*
44	*Rr*	*rr*	*RR*	92	*Rr*	*rr*	*Rr*
45	*Rr*	*rr*	*rr*	93	*Rr*	-	*rr*
46	*RR*	*rr*	*rr*	94	*RR*	-	*rr*
47	*Rr*	*Rr*	*rr*	95	*Rr*	*Rr*	*RR*
48	*rr*	*rr*	*rr*	96	*rr*	*RR*	*RR*

RR= homozygote resistance, *Rr*= heterozygote, *rr*= sensitive genotypes, -= not detected.

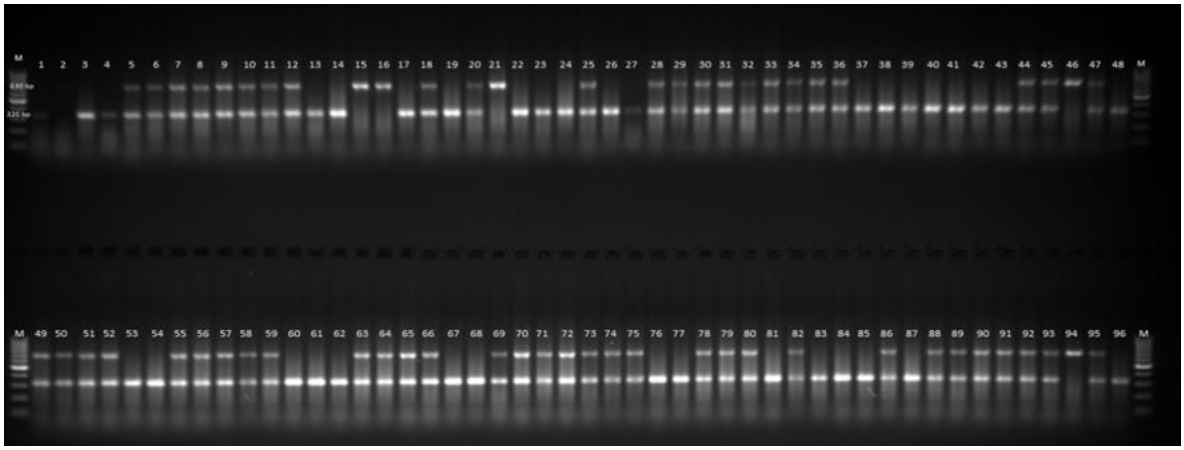

Figure 1. PCR reactions of different tomato lines for Ty3P6-25. M= 100 bp DNA marker, 1-96= tomato lines.

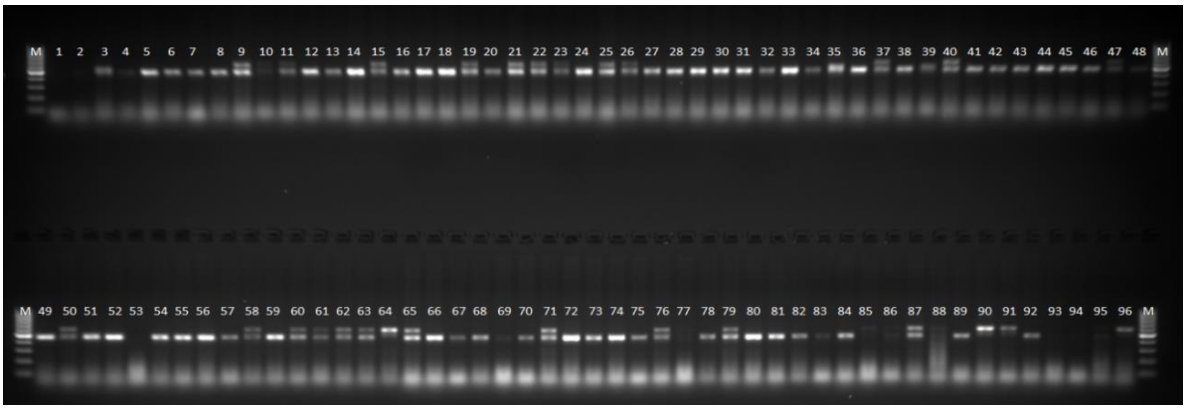

Figure 2. PCR reactions of different tomato lines for Sw5-2. M= 100 bp DNA marker, 1-96= tomato lines.

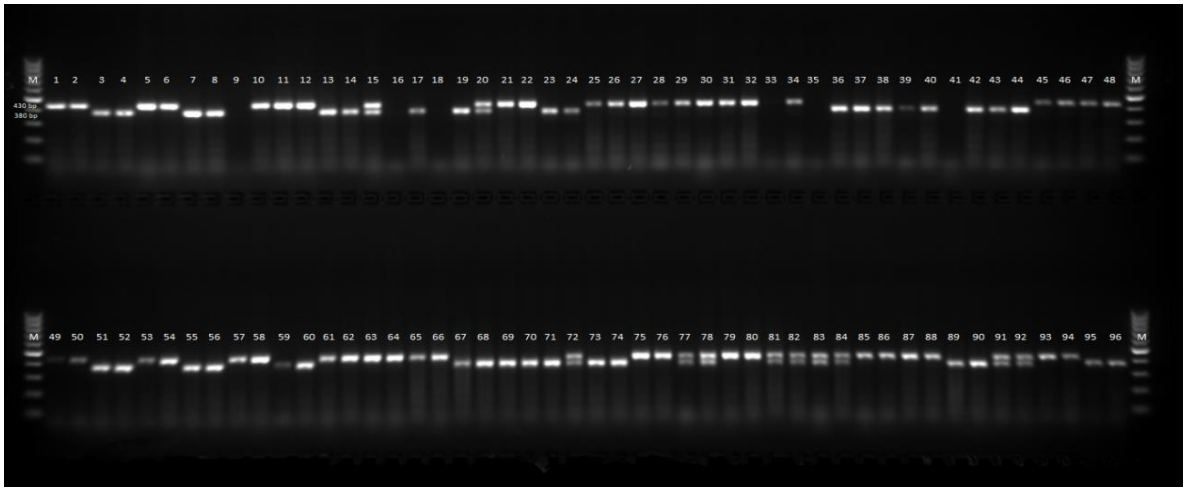

Figure 3. PCR reactions of different tomato lines for Mi23. M= 100 bp DNA marker, 1-96= tomato lines.

4. Discussion

Evaluation of the results of this study showed that molecular markers against Tomato Spotted Wilt virus (*TSWV*), Tomato Yellow Leaf Curl Virus (*TYLCV*) and root knot nematodes (*Meloidogyne* spp.) can be readily used for breeding existing tomato lines. Therefore, molecular markers (*Sw5*-2) determining resistance to Tomato Spotted Wilt Virus (*TSWV*), resistance to Tomato Yellow Leaf Curl Virus (*TYLCV*) (*Ty*3 P6-25) and root-knot nematodes (*Meloidogyne* spp.) (*Mi*23) should be used in various breeding programs to develop resistant cultivars to the above diseases. This will provide both the opportunity to test more material and increase the success rate by reducing the duration of breeding programs.

Although plant lines resistant to diseases have been determined in breeding programs with molecular markers, pathogenicity tests are essential for a safer resistance. Therefore, testing different genotypes of the pathogen against plants that have been identified as resistant may provide a more accurate and stable resistance against genotypes of the pathogen from

different geographic areas. For this purpose, in the next step of this study, tomato lines determined to be resistant by molecular markers will be tested *in vivo* against different genotypes of Tomato Spotted Wilt Virus (*TSWV*), Tomato Yellow Leaf Curl Virus (*TYLCV*) and root knot nematodes (*Meloidogyne* spp.).

The use of molecular markers and pathogenicity tests together in determining the resistance reactions against diseases is of great importance in terms of the reliability and sustainability of the resistance obtained. In such studies, it is essential to have different strains or genotypes of pathogens for pathogenicity tests.

5. Conclusion

In this study, determination of the resistance of tomato lines against Tomato Spotted Wilt Virus (*TSWV),* Tomato Yellow Leaf Curl Virus (*TYLCV*) and root knot nematodes (*Meloidogyne* spp.), and further studies confirming this resistance by pathogenicity tests, then will be carried out. Some of the tomato lines have resistance to all biotic factors used in this study. This will allow the development of new tomato varieties resistant to diseases. Based on the results of this study, it will also be possible to developed three biotic factor resistances in the same tomato variety.

Author Contributions

Concept: H.B. (100%), Design: H.B. (100%), Supervision: O.K. (100%), Data collection and/or processing: M.M. (50%) and R.İ. (50%), Data analysis and/or interpretation: H.B. (100%), Literature search: H.B. (25%), O.K. (25%), R.İ. (25%) and M.M. (25%), Writing: H.B. (100%), Critical review: H.B. (100%). Submission and revision H.B. (100%). All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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