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Screening of Gene Based Markers with 23 Genotypes in Tomato (*Solanum lycopersicum* L)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The investigation was conducted at SKL Telangana State Horticultural University, Rajendranagar in *Kharif*, 2018 using 23 tomato genotypes. Screening with Ty-1 (P6-6) marker, only one genotype i.e. AVTO-1219 showed resistant band. Screening with Ty-2 marker, only 2 genotypes viz., AVTO-1219 and AVTO-9804 showed resistant bands. Screening with Ty-1 (SSR 47) marker, 3 genotypes i.e. AVTO-1219, AVTO-9803 and AVTO-9804 showed resistant bands. Using Fw-Z 1063 marker,

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10 genotypes viz., EC-615055, EC-620428, AVTO-1219, EC-620378, EC-620389, EC-620394, EC-620422, EC-631369, EC-620503 and AVTO-9803 showed resistant bands. At molecular level, these ten genotypes showed resistant bands and these genotypes contain I-2 genes.

Keywords: Genetic diversity; gene specific primers; fusarium wilt; tomato yellow leaf curl virus and tomato spotted wilt virus.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L., which is formerly named as *Lycopersicon esculentum* L.), have been originated from South America, in the Andes Mountains of Peru, Ecuador, and Chile, is nowadays one of the most popular and widely grown plants in Solanaceae family. In addition tomato is an pre-eminent model system for genetic studies in plants.

"Few of the studies have been carried out to investigate genetic diversity accessions in tomato which are collected from primary and secondary centres using morphological, biochemical and molecular approaches" [1,2,3,4,5].

The tomato production is caused by many of the factors *i.e.,* abiotic stresses like heat, drought, salinity stresses and biotic stress like viral, fungal and bacterial infections. The major viral pathogens like Yellow Leaf Curl Virus (TYLCV), Tomato Spotted Wilt Virus (TSWV), Tomato mosaic virus (ToMV), Tobacco Mosaic Virus (TMV), Cucumber Mosaic Virus (CMV) and Fusarium wilt (*Fusarium oxysporum*), Verticillium wilt (*Verticillium* spp.), Early blight (*Alternaria solani*), Late blight (*Phytophthora infestans*) are some of fungal diseases and bacterial diseases like Bacterial canker, Bacterial wilt effect the tomato yield. Among all of these Tomato spotted wilt, which is a major viral disease caused by TSWV belonging to Tospovirus. It has a host range exceeding 1000 plant species which include tomato, bean, lettuce and many others and transmitted by small insects called thrips. It affects many of the trade industry causing massive losses not only in tomato but also in many of the vegetable and ornamental crops globally [6,7].

"There are extensive worldwide crop losses in tomato production due to multiple Fusarium species" [8]. "The pathogen will resides in the soil, infecting plants mainly through their roots and crown portions. Different species of Fusarium are also associated with the wilt of tomatoes such as *Fusarium verticillioides*, F. *oxysporum*, and F. *equiseti"* [9]. "They can infect tomatoes mainly through both roots and crown area at any growth stage. Some species, such as *Fusarium oxysporum*, infect vascular bundles, causing infected plants show an early wilting syndrome due to stress" [10,11]. "Fusarium wilt disease is more common in acidic sandy soils. For a period of up to ten years the wilt pathogen can reside in infested soil. Soil with very warm (34˚C) or cool (17-20˚C) temperatures slow down wilt development" [12].

"Tomato yellow leaf curl disease is one of the major limiting factor for tomato production worldwide. It is caused by a whitefly-transmitted Begomovirus (family Geminiviridae). Since this whitefly vector is difficult to control and tomato yellow leaf curl resistance is absent in the cultivated tomato gene pool, emphasis was given to identify host resistance against the TYLCV disease in wild tomato species such as S. *chilense*, S. *habrochaites* and S. *peruvianum"* [13,14,15, 16,17,18,19].

In this study 23 genotypes evaluated with Fusarium wilt, Tomato yellow leaf curl virus and Tomato spotted wilt virus gene specific primers and identified the resistant genotypes. These Molecular markers are used for the marker assisted selection to improve the traits like yield, biotic and abiotic stress resistance, due to the demand and economical importance of the tomato. Henceforth, there is necessity to develop new genotypes with high yield and resistance characteristics to biotic and abiotic stress. It will be further useful to advancing the MAS.

2. MATERIALS AND METHODS

2.1 Plant Material

A total of 23 tomato genotypes (Table 1) was maintained at the PG Research Block, Department of Vegetable Science, College of Horticulture, Rajendranagar, SKLTSHU, Hyderabad during *Kharif*, 2018.

Table 1. List of genotypes and their sources [18]

S.No.	Genotypes	Source
1.	EC-615055	NBPGR, Hyderabad
2.	EC-620463	NBPGR, Hyderabad
3.	EC-620428	NBPGR, Hyderabad
4.	AVTO-1219	WVC, Taiwan, China
5.	EC-620378	NBPGR, Hyderabad
6.	EC-620382	NBPGR, Hyderabad
7.	EC-620389	NBPGR, Hyderabad
8.	EC-620395	NBPGR, Hyderabad
9.	EC-620406	NBPGR, Hyderabad
10.	EC-620427	NBPGR, Hyderabad
11.	EC-620394	NBPGR, Hyderabad
12.	EC-620422	NBPGR, Hyderabad
13.	EC-631369	NBPGR, Hyderabad
14.	EC-631379	NBPGR, Hyderabad
15.	EC-620503	NBPGR, Hyderabad
16.	AVTO-9803	WVC, Taiwan, China
17.	AVTO-9804	WVC, Taiwan, China
18.	AVTO-1002	WVC, Taiwan, China
19.	AVTO-0101	WVC, Taiwan, China
20.	Pusa Ruby	IARI, New Delhi
21.	PKM-1	Periyakulam, TNAU
22.	Pant bahar	GBPUAT, Uttarakhand
23.	Arka vikas	IIHR, Bengaluru

Table 2. DNA markers used for selection of resistant tomato genotypes against Fusarium wilt, tomato yellow leaf curl virus and TSWV

Primer	Sequence $5^{I} \longrightarrow 3^{I}$	Chromosome number	Annealing Temperature
Fw-Z 1063	F:ATTTGAAAGCGTGGTATTGC R:CTTAAACTCACCATTAAATC	11	55^0C
Ty1 (P6-6)	F:CAATTTATAGGTGTTTTTGGGACATC R:GTTCACACTTGGCCAATGCTTACG	6	55^0C
Ty 2	F:CCCACCACTCAAGGCAAAGTAAGA R:AAACAACGACACACCGACCGATA	11	56.4^0C
Ty 1 (SSR 47)	F:TCCTCAAGAAATGAAGCTCTGA R:CCTTGGAGATAACACCACAA	6	55^0C
Sw-5	F:CGGAACCTGTAACTTGACTG R:GAGCTCTCATCCATTTTCCG	9	55^0C

2.2 DNA Isolation and SSR Analysis

Fresh mature leaf samples was collected from 23 genotypes and DNA was isolated by following modified CTAB method (Murray and Thompson 1980). The quality and quantity of isolated DNA was checked using 0.8% agarose gel electrophoresis and Biophotometer (Eppendorff). Finally DNA was diluted using nuclease free water to a concentration of 50ng/ul to perform further PCR analysis. Five gene specific primers against Fusarium wilt, Tomato yellow leaf curl virus and TSWV (Table 2). The PCR conditions were standardized by modifying annealing temperature according to the primer. The amplified products were separated in a 3% agarose gel along with the marker (100-bp ladder) in 0.5 Tris– Boric acid–EDTA (TBE) buffer. The resolved PCR bands were documented using gel documentation unit.

2.3 Data Analysis

The bands which are present were scored as resistance and absence were scored as susceptible respectively.

3. RESULTS AND DISCUSSION

3.1 Screening of Tomato Germplasm Accessions Using Gene Specific Markers

In this study five gene specific primers were used to screen the 23 tomato germplasm accessions for their resistance against Fusarium wilt, Tomato Yellow leaf curl disease and TSWV disease.

3.1.1 PCR based screening with Fw-Z 1063 marker

Out of 23 genotypes, 10 genotypes viz., EC-615055, EC-620428, AVTO-1219, EC-620378, EC-620389, EC-620394, EC-620422, EC-631369, EC-620503 and AVTO-9803 showed resistant bands (Fig. 1). At molecular level, these ten genotypes showed resistant bands and these genotypes contain *I-2* genes.

3.1.2 Screening 23 genotypes of tomato with Ty-1, Ty-2 markers

All the 23 tomato genotypes were screened for Tomato yellow leaf curl disease (TYLCD), which is a global constraint to tomato production (Moriones and Navas-Castillo, 2000). Thus, the identification of resistance sources against TYLC will be useful. For this all the 23 genotypes were screened with Ty-1 and Ty-2 gene specific markers.

3.1.3 Screening with Ty-1(P6-6) marker

All the twenty three genotypes of tomato were screened with Ty-1 gene based marker to identify Ty-1 gene loci. As a result, out of 23 genotypes only one genotypes i.e. AVTO1219 was showed the resistance loci at desired product size. Thus indicating that resistance nature of AVTO1219 against TYLC disease due to the presence of Ty-1 loci on chromosome number 6 (Fig. 2).

Marker	Sequence
Fw-Z 1063	F: ATTTGAAAGCGTGGTATTGC
	R: CTTAAACTCACCATTAAATC

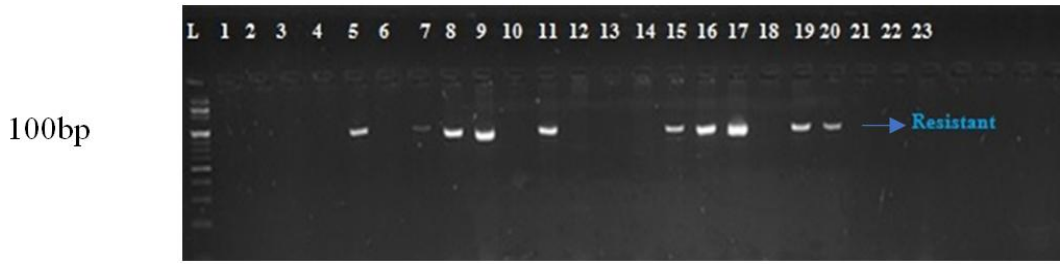

Fig. 1. PCR based screening with Fw-Z 1063 marker

Marker	Sequence
Ty-1-P6-6	F: CAATTTATAGGTGTTTTTGGGACATC
	R: GTTCACACTTGGCCAATGCTTACG

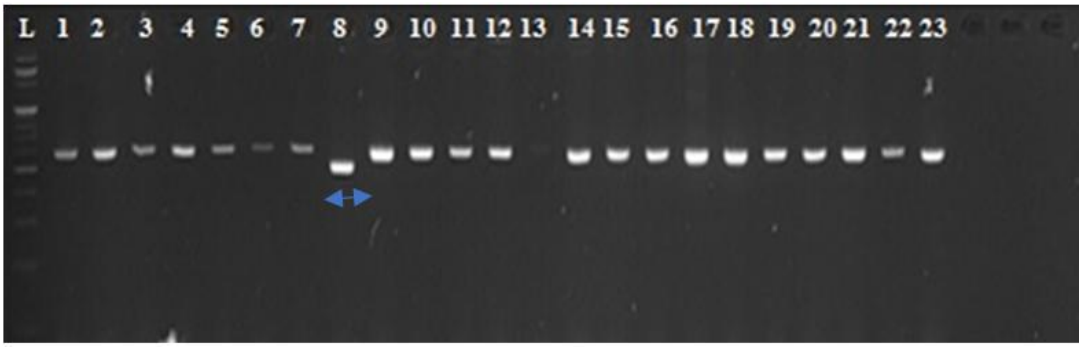

Fig. 2. Screening 23 genotypes of tomato with Ty-1, Ty-2 markers

3.1.4 Screening with Ty-2 marker

Twenty three genotypes of tomato were also screened with Ty-2 gene based marker to identify the presence of Ty-2 loci, which confers the resistance against TYLCV (Fig. 3). The sequence of Ty-2 marker is:

Marker	Sequence
Ty-2	F: CCCACCACTCAAGGCAAAGTAAGA
	R: AAACAACGACACACCGACCGATA

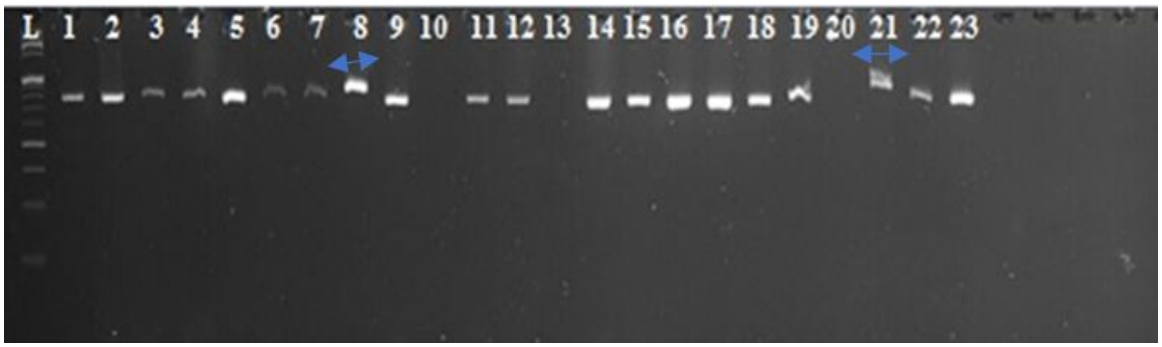

Fig. 3. Screening with Ty-2 marker

As a result, out of 23 genotypes, only two genotypes i.e. AVTO1219 and AVTO9804, which indicates the resistance nature of AVTO-1219 and AVTO-9804 against TYLC disease due to the presence of Ty-2 loci on chromosome number 11.

Thus it is also confirmed that AVTO 1219 is resistance to TYLC with the presence of Ty-1 and Ty-2 loci. Whereas the other genotype AVTO9804 was also resistance against TYLC with single loci Ty-2.The remaining genotypes in this study was not showed any desired bands with respect to Ty-1 and Ty-2 indicates all are susceptible to TYLC.

3.1.5 Screening with Ty 1 (SSR 47) marker

Twenty three genotypes of tomato were screened with gene based marker Ty 1 (SSR 47) Out of 23 genotypes 3 genotypes viz., AVTO 1219, AVTO 9803 and AVTO 9804 showed resistant nature against TYLC disease due to the presence of Ty-2 loci on chromosome number 6. The sequence of Ty 1 (SSR 47) marker is

Marker	Sequence
Ty 1 (SSR 47)	F:TCCTCAAGAAATGAAGCTCTGA
	R:CCTTGGAGATAACACCACAA

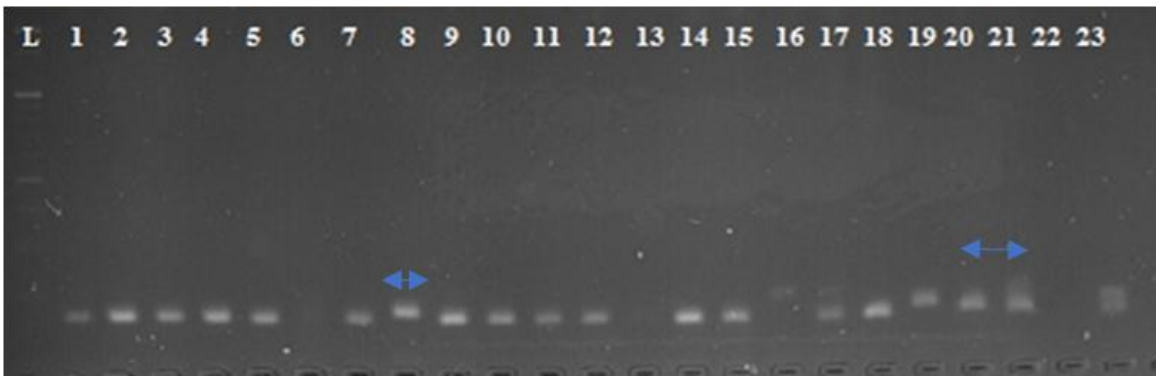

Fig. 4. Screening with Ty 1 (SSR 47) marker

Marker	Sequence
Sw-5	F:CGGAACCTGTAACTTGACTG R:GAGCTCTCATCCATTTTCCG

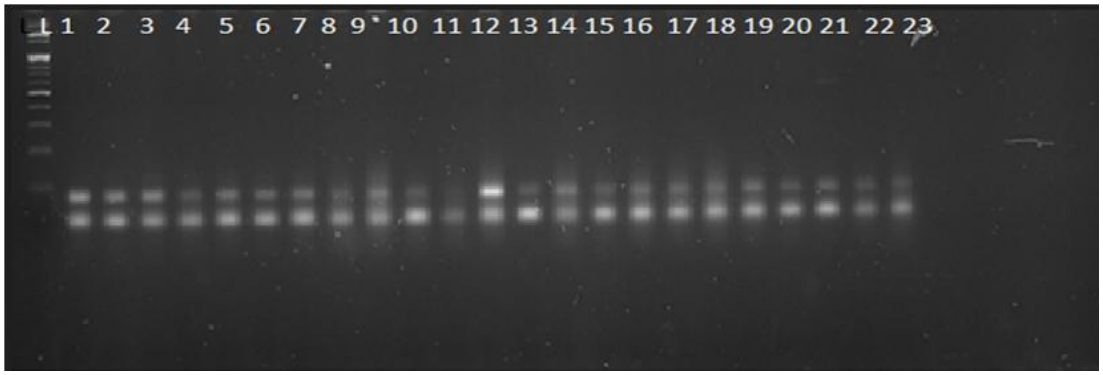

Fig. 5. Screening with Sw-5 gene based marker

3.1.6 Screening with Sw-5 gene based marker

All the 23 genotypes were also screened with Sw-5 gene based marker to check the resistance against Tomato spotted wilt virus (TSWV). As a dominant marker, the desired band presence at 540 bp indicates the presence of Sw-5 gene in the respective genotype. As a result, amplification was not observed in any of the single genotypes. Thus it was clear that all the genotypes were susceptible to TSWV due to the absence of Sw-5 gene.

4. CONCLUSION

Hence in the present study, Fw-Z EC-615055, EC-620428, AVTO-1219, EC-620378, EC-620389, EC-620394, EC-620422, EC-631369, EC-620503 and AVTO-9803 with I-2 genes; AVTO-1219 with Ty-1(P6-6) gene, only 2 genotypes viz., AVTO-1219 and AVTO-9804 with Ty-2gene , 3 genotypes i.e. AVTO-1219, AVTO-9803 and AVTO-9804 with Ty-1(SSR 47) gene may be used for disease resistance varieties or used as resistant parents and exploited as in breeding programmes.

5. FUTURE SCOPE

The present experiment would help in creating a base line for future work. The resistant genes can be further useful for marker assisted breeding program for the improvement of tomato crop.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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