

# Molecular characterization of markers linked to *Tomato spotted wilt virus* and *Tomato mosaic virus* resistance loci in tomato

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## ABSTRACT

The *Tomato spotted wilt virus* (TSWV) and *Tomato mosaic virus* (ToMV) are among the most common viral diseases that negatively affect the tomato crop. The use of tomato genotypes containing virus resistance genes is considered the best method for virus control. In this study, attempts were made to identify the *Sw-5* and *Sw-5b* as well as *Tm-1* and *Tm-2* and its allele *Tm-2<sup>2</sup>* loci known to influence resistance to the TSWV and ToMV, respectively, in 19 tomato genotypes using molecular markers. In this work, 18 tomato genotypes were found to be resistant to the TSWV. These lines have dominant alleles with homozygous or heterozygous *Sw5* or *Sw5b* or both. Also, seven lines were resistant to the ToMV, which have dominant or recessive alleles for *Tm-1* or *Tm-2* or *Tm-2<sup>2</sup>* or *tm-2<sup>2</sup>*, separately or mixed. In general, phenotypic results were highly matched with genotypic data, but gene-based markers displayed clearer results than biological tests; e.g., the presence of dominant and recessive alleles of the resistance gene can be identified readily in tomato genotypes. Therefore, the originality of this work is the discovery of donor parents for developing tomato genotypes resistant to both the TSWV and ToMV in tomato breeding programs or the genetic improvement of *Solanum lycopersicum* L. lines with pyramided genes for pathogen resistance by marker-assisted selection.

## 1. INTRODUCTION

The *Tomato spotted wilt virus* (TSWV) and *Tomato mosaic virus* (ToMV) are two of the most dangerous viral infections that threaten tomato (*Solanum lycopersicum* L.) crops around the world [1,2]. Genetic resistance to viral diseases by using resistance genes has been applied for 80 years to decrease crop losses. Up to now, several resistance loci have been found in different crops and wild species [3]. The TSWV is a member of the genus *Tospovirus* in the family *Bunyaviridae*. TSWV symptoms are necrotic spots, curling, bronzing, and stunting of plants [4]. The TSWV infects both monocotyledons and dicotyledons [5]. TSWV resistance sources were identified in different tomato genotypes, e.g., *Solanum habrochaites* and *S. habrochaites* var. *glabratum* (“PI134417” and “LA1223”) [1]. Up to now, many resistance loci to the TSWV have been defined, namely, *Sw1a*, *Sw1b*, *sw2*, *sw3*, *sw4*, *Sw-5* (*Sw-*

*5a* to *Sw-5e*), *Sw-6*, and *Sw-7* [1,6–8]. *Sw-5* is one of the TSWV resistance alleles which has been used to generate TSWV-resistant tomato cultivars. *Sw-5*, which is found on chromosome 9 of *S. peruvianum*, is known to give resistance to *Tospoviruses* such as the TSWV [9–11]. The *Sw-5* protein is made up of three domains: a coiled-coil (CC) domain, a nucleotide-binding site (NBS), and a leucine-rich repeat (LRR) domain [7].

The ToMV belongs to the genus *Tobamovirus*, which is one of the family *Virgaviridae*. Three ToMV resistance alleles have been introduced into domesticated tomatoes: *Tm-1*, *Tm-2*, and *Tm-2<sup>2</sup>*. The tomato gene *Tm-1* has been discovered in *S. habrochaites* “PI126445” mapped to chromosome 2 [12,13]. It has been known that resistance to the ToMV is due to inhibition of movement of the virus into the plant cells. The *Tm-2* and *Tm-2<sup>2</sup>* resistance alleles conferred a higher level of resistance than the *Tm-1* allele in a wild-type tomato, *S. peruvianum* [14,15]. Furthermore, tomato plants with *Tm-2* or *Tm-2<sup>2</sup>* show a hypersensitive response to the ToMV [16,17]. *Tm-2* and its allele *Tm-2<sup>2</sup>* have been found near the centromere of chromosome 9 [18]. Besides, the resistance locus *Tm-2<sup>2</sup>* is more durable than *Tm-2* [19]. Therefore, the *Tm-2<sup>2</sup>* resistance locus is both economically and practically important. It is used in

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tomato breeding programs as a source of ToMV resistance. *Tm-2* and *Tm-2<sup>2</sup>* are resistance (*R*) loci in the plant host which encode members of the CC/nucleotide-binding-ARC/LRR protein family [20]. *Tm-2<sup>2</sup>* and *Tm-2* have seven nucleotide variations in their open reading frames, resulting in four amino acid changes at the protein level. Two of these distinctions belong to the NBS domain, whereas the other two belong to the LRR domain [21].

Here in this investigation, a set of allele-specific markers were used to identify the resistance alleles *Sw-5* and *Sw-5b* as well as *Tm-1*, *Tm-2*, and *Tm-2<sup>2</sup>*, responsible for resistance to the TSWV and ToMV, respectively, in 19 tomato genotypes. Therefore, the functional markers can be used as a powerful tool in tomato breeding programs for TSWV and ToMV resistance.

## 2. MATERIALS AND METHODS

### 2.1. Plant Materials

Nineteen tomato lines, including accessions and commercial cultivars, were utilized in this investigation, as mentioned in Table 1. Each tomato seed was cultivated in a pot containing peat moss:vermiculite:sand in a ratio of 1:1:1. The pots were kept in a glasshouse in 27°C light/16°C dark, a photoperiod of 16 hours light:8 hours dark cycle, and 68%–75% relative humidity [22,23].

### 2.2. Virus Resistance Tests

#### 2.2.1. Source of virus isolates

The TSWV and ToMV isolates were obtained from the Virology Laboratory, Department of Agricultural Microbiology, Faculty of Agriculture, University of Ain Shams. The TSWV and ToMV were maintained on *Nicotiana tabacum* cv. White Burley and *Datura metel* L. plants, respectively (Figs. 1 and 2).

#### 2.2.2. Virus inoculation

One-month-old tomato lines cultivated in the glasshouse were mechanically inoculated with TSWV- or ToMV-infected tomato sap according to Green [24]. Virus symptoms were recorded for

4 weeks after inoculation. These materials were evaluated in two successive seasons, 2019–20 and 2020–21, in the greenhouse.

### 2.3. Evaluation of TSWV and ToMV Infection Under Greenhouse Conditions

Disease rating scales of 0 to 4 were used according to Hutton and Scott [25].

#### 2.3.1. Serological assay using BIOREBA immunostrips for detection of TSWV and ToMV

All tomato plants inoculated with the TSWV or ToMV were tested for the presence of virus by AgriStrips using the virus-specific polyclonal. The TSWV and ToMV AgriStrips are a one-step assay that was developed and manufactured by BIOREBA AG, Reinach, Switzerland.

#### 2.3.2. Isolation of DNA

Using a DNA purification kit (Bio Basic, Inc., Markham, Canada), DNA was isolated from tissues of 19 tomato genotypes.

#### 2.3.3. Polymerase chain reaction (PCR) amplification of resistance alleles

PCR-based markers [sequence characterized amplified regions (SCAR) and amplification-refractory mutation system (ARMS)] were carried out as mentioned below. The conditions were adjusted in 25 µl reactions containing 2.5 µl 2.5 mM dNTPs, 5 µl 5× buffer, 2.5 µl 2.5 mM MgCl<sub>2</sub>, 0.1 µl (0.5 units) *Taq* DNA polymerase (Promega Corp., Madison, WI), 2.5 µl of each forward and reverse primer at 10 µM, 1 µl of DNA extract, and 8.9 µl dH<sub>2</sub>O. PCR cycles were 94°C for 4 minutes, 35 cycles of 94°C for 30 seconds, annealing temperature (Table 2) for 1 minute, and 72°C for 1.5 minutes. These cycles were followed by 72°C for 10 minutes, and then the reaction was held at 4°C. PCR reactions were performed in the thermocycler (Biometra, biomedizinische Analytik GmbH).

#### 2.3.4. Gel electrophoresis

All the PCR products were separated on 1% agarose gel electrophoresis in a 1×TBE (Tris/Borate/EDTA buffer), stained with

**Table 1:** Tomato genotypes used in this study.

No.	Genotype	Source	No.	Genotype	Source
1	<i>S. hirsutum</i> 24036	CGN <sup>a</sup>	11	<i>S. chilense</i> 56139	CGN
2	<i>S. galapagense</i> 0317	TGRC <sup>b</sup>	12	<i>S. lycopersicon</i> cv. Super Marmande	Egypt <sup>c</sup>
3	<i>S. neoricki</i> 0247	TGRC	13	<i>S. lycopersicon</i> cv. Strain B F1	Egypt
4	<i>S. arcanum</i> 1346	TGRC	14	<i>S. corneliomulleri</i> 1283	TGRC
5	<i>S. corneliomulleri</i> 1274	TGRC	15	<i>S. habrochaites</i> 1739	TGRC
6	<i>S. pennellii</i> 1733	TGRC	16	<i>S. pimpinellifolium</i> 1279	TGRC
7	<i>S. huaylasense</i> 1358	TGRC	17	<i>S. pimpinellifolium</i> 1332	TGRC
8	<i>S. pimpinellifolium</i> 1342	TGRC	18	<i>S. pennellii</i> 2963	TGRC
9	<i>S. peruvianum</i> 1333	TGRC	19	<i>S. pennellii</i> 1942	TGRC
10	<i>S. habrochaites</i> 1352	TGRC			

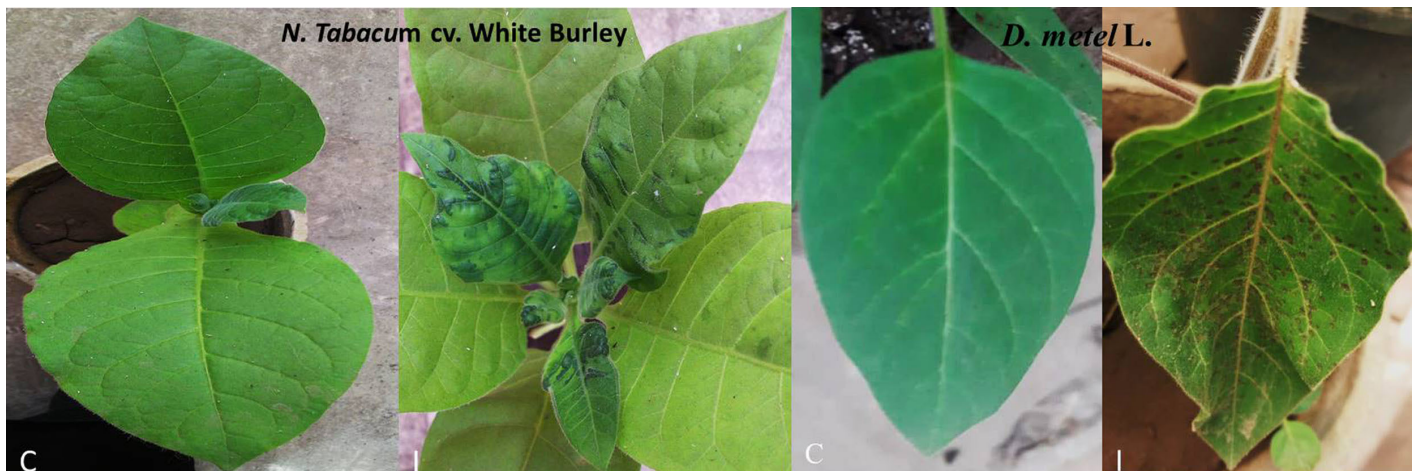
<sup>a</sup> CGN = Centre for Genetic Resources, Netherlands (<http://www.wur.nl>).

<sup>b</sup> TGRC = Tomato Genetics Resource Center (TGRC), Department of Plant Sciences, University of California, Davis, CA 95616 (<http://tgrc.ucdavis.edu>).

<sup>c</sup> Commercial cultivar was purchased from Egyptian Company for Seeds, Oils and Chemicals, Egypt.



**Figure 1:** Photographs of *N. tabacum* cv. White Burley leaf after inoculation with TSWV isolate showing local necrotic lesions (I), compared with the healthy control (C).



**Figure 2:** *N. tabacum* cv. White Burley (left) and *D. metel* L. (right) inoculated with ToMV, appeared (severe mosaic and malformation), (necrotic local lesions) symptoms, respectively. C = healthy control; I = inoculated plants.



**Table 2:** Sequence of primers used in this study.

Primer name	Marker name	Disease name	R-gene <sup>c</sup>	Chromosome no.	Single nucleotide sequence (5'-3')	Annealing temperature (AT) <sup>°C</sup>	Molecular size of PCR product (bp)	References
Sw5-2 SCAR F					AATTAGGTTCTTGAAGCCCATCT			
	SCAR	TSWV	<i>Sw5</i>	9		50	$R^d = 574$ $S^e = 464$ or 510	[26]
Sw5-2 SCAR R					TTCCGCATCAGCCAATAGTGT CGGAACCTGTAACCTGACTG			
Sw5b SCAR F								
Sw5b SCAR R	SCAR <sup>a</sup>	TSWV	<i>Sw5b</i>	9		56	$R = 541$	[27]
Tm-1 SCAR F					GAGCTCTCATCCATTTTCCG GGTGCTCCGTCGATGCAAAGTGCA			
	SCAR	ToMV	<i>Tm-1</i>	2		60	$R = 1,400$ Other = 92	[28]
Tm-1 SCAR R					GTGCTCCGTAGACATAAAATCTA CTCATCAAGCTTACTCTAGCCTACTTTAGT			
Tm-2 <sup>2</sup> ARMS F								
Tm-2 <sup>2</sup> ARMS R	ARMS <sup>b</sup>	ToMV	<i>Tm-2</i> or <i>Tm-2<sup>2</sup></i> or <i>tm-2<sup>2</sup></i>	9		55	$R = 179$ ( <i>Tm-2</i> or <i>Tm-2<sup>2</sup></i> ) $S = 382$ ( <i>tm-2</i> )	[21,29]
					CTGCCAGTATATAACGGTCTACCG			

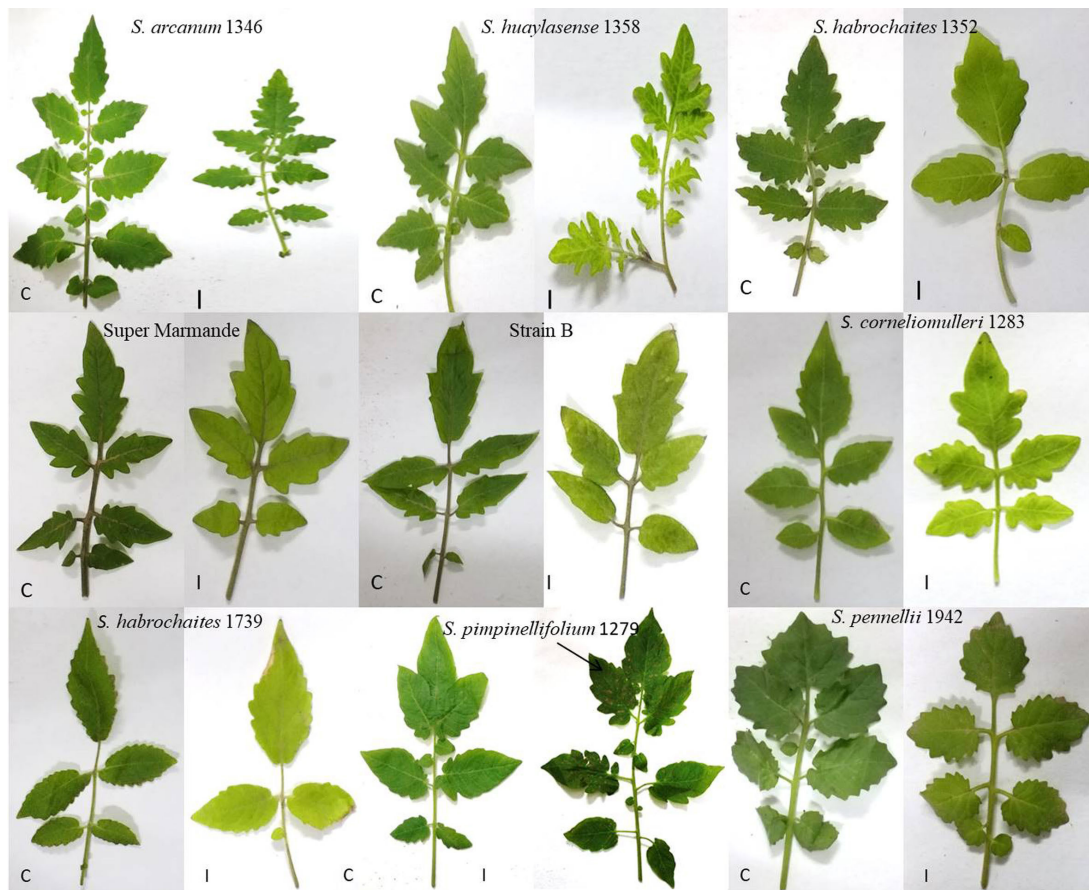
<sup>a</sup>SCAR = Sequence characterized amplified region.

<sup>b</sup>ARMS = Amplification refractory mutation system.

<sup>c</sup>Resistance genes of disease.

<sup>d</sup>R = Resistant.

<sup>e</sup>S = Susceptible.



**Figure 3:** Phenotype of inoculated tomato genotypes three weeks after inoculation with TSWV virus. C: control, I: inoculated plants with TSWV. *S. huaylasense* 1358, *S. habrochaites* 1352 and 1739, Super Marmande, Strain B, and *S. pennellii* 1942 displayed mild mosaic, *S. arcanum* 1346 gave small leaf size, *S. corneliomulleri* 1283 appeared yellowing and *S. pimpinellifolium* 1279 showing necrotic spot. The arrow points to necrotic spots.

**Table 3:** Tomato genotypes used to evaluate gene-based markers for resistance against TSWV and ToMV.

No.	Accession/Cultivar	Viruses, resistance genes and DNA markers							
		TSWV <sup>a</sup>		ToMV <sup>b</sup>		TSWV ( <i>Sw5</i> )	TSWV ( <i>Sw5b</i> )	ToMV ( <i>Tm-1</i> )	ToMV ( <i>Tm-2</i> or <i>tm-2</i> )
		Disease severity	Phenotype	Disease severity	Phenotype	Sw5 SCAR	Sw5b SCAR <sup>c</sup>	Tm-1 SCAR	Tm-2 <sup>2</sup> ARMS <sup>d</sup>
1	<i>S. hirsutum</i> 24036	2	Moderately resistant	1	Resistant	<i>Rr</i> <sup>e</sup> ( <i>Sw5sw5</i> )	<i>rr</i> <sup>e</sup> ( <i>sw5bSw5b</i> )	-	-
2	<i>S. galapagense</i> 0317	2	Moderately resistant	1	Resistant	<i>rr</i> ( <i>sw5sw5</i> )	<i>RR</i> <sup>f</sup> ( <i>Sw5bSw5b</i> )	-	-
3	<i>S. neorickii</i> 0247	1	Resistant	0	Highly resistant	<i>RR</i> ( <i>Sw5Sw5</i> )	<i>RR</i> ( <i>Sw5bSw5b</i> )	-	<i>rr(tm-2<sup>2</sup>tm-2<sup>2</sup>)</i>
4	<i>S. arcanum</i> 1346	3	Moderately susceptible	1	Resistant	<i>rr</i> ( <i>sw5sw5</i> )	<i>RR</i> ( <i>Sw5bSw5b</i> )	<i>RR (Tm-1Tm-1)</i>	-
5	<i>S. corneliomulleri</i> 1274	1	Resistant	0	Highly resistant	<i>rr</i> ( <i>sw5sw5</i> )	<i>RR</i> ( <i>Sw5bSw5b</i> )	-	-
6	<i>S. pennellii</i> 1733	1	Resistant	0	Highly resistant	<i>RR</i> ( <i>Sw5Sw5</i> )	<i>RR</i> ( <i>Sw5bSw5b</i> )	-	-
7	<i>S. huaylasense</i> 1358	1	Resistant	0	Highly resistant	<i>rr</i> ( <i>sw5sw5</i> )	<i>RR</i> ( <i>Sw5bSw5b</i> )	<i>RR (Tm-1Tm-1)</i>	-
8	<i>S. pimpinellifolium</i> 1342	2	Moderately resistant	2	Moderately resistant	<i>rr</i> ( <i>sw5sw5</i> )	<i>RR</i> ( <i>Sw5bSw5b</i> )	-	<i>rr(tm-2<sup>2</sup>tm-2<sup>2</sup>)</i>
9	<i>S. peruvianum</i> 1333	0	Highly resistant	1	Resistant	<i>RR</i> ( <i>Sw5Sw5</i> )	<i>RR</i> ( <i>Sw5bSw5b</i> )	<i>RR (Tm-1Tm-1)</i>	-
10	<i>S. habrochaites</i> 1352	1	Resistant	3	Moderately susceptible	<i>rr</i> ( <i>sw5sw5</i> )	<i>RR</i> ( <i>Sw5bSw5b</i> )	<i>RR (Tm-1Tm-1)</i>	<i>RR (Tm-2 Tm2 or Tm-2<sup>2</sup> Tm-2<sup>2</sup>)</i>
11	<i>S. chilense</i> 56139	0	Highly resistant	0	Highly resistant	<i>Rr</i> ( <i>Sw5sw5</i> )	<i>RR</i> ( <i>Sw5bSw5b</i> )	-	-
12	<i>S. lycopersicon</i> cv. Super Marmande	1	Resistant	0	Highly resistant	<i>RR</i> ( <i>Sw5Sw5</i> )	<i>RR</i> ( <i>Sw5bSw5b</i> )	-	<i>rr(tm-2<sup>2</sup>tm-2<sup>2</sup>)</i>
13	<i>S. lycopersicon</i> cv. Strain B F1	3	Moderately susceptible	1	Resistant	<i>RR</i> ( <i>Sw5Sw5</i> )	<i>RR</i> ( <i>Sw5bSw5b</i> )	-	<i>rr(tm-2<sup>2</sup>tm-2<sup>2</sup>)</i>
14	<i>S. corneliomulleri</i> 1283	4	Susceptible	0	Highly resistant	<i>RR</i> ( <i>Sw5Sw5</i> )	<i>RR</i> ( <i>Sw5bSw5b</i> )	<i>RR (Tm-1Tm-1)</i>	-
15	<i>S. habrochaites</i> 1739	1	Resistant	0	Highly resistant	<i>Rr</i> ( <i>Sw5sw5</i> )	<i>RR</i> ( <i>Sw5bSw5b</i> )	<i>RR (Tm-1Tm-1)</i>	<i>RR (Tm-2 Tm2 or Tm-2<sup>2</sup> Tm-2<sup>2</sup>)</i>
16	<i>S. pimpinellifolium</i> 1279	2	Moderately resistant	0	Highly resistant	<i>rr</i> ( <i>sw5sw5</i> )	<i>RR</i> ( <i>Sw5bSw5b</i> )	-	<i>rr (tm-2<sup>2</sup>tm-2<sup>2</sup>)</i>
17	<i>S. pimpinellifolium</i> 1332	3	Moderately susceptible	2	Moderately resistant	<i>rr</i> ( <i>sw5sw5</i> )	<i>rr</i> ( <i>sw5bSw5b</i> )	-	-
18	<i>S. pennellii</i> 2963	3	Moderately susceptible	0	Highly resistant	<i>rr</i> ( <i>sw5sw5</i> )	<i>RR</i> ( <i>Sw5bSw5b</i> )	<i>RR (Tm-1Tm-1)</i>	-
19	<i>S. pennellii</i> 1942	1	Resistant	0	Highly resistant	<i>RR</i> ( <i>Sw5Sw5</i> )	<i>RR</i> ( <i>Sw5bSw5b</i> )	-	<i>rr(tm-2<sup>2</sup>tm-2<sup>2</sup>)</i>

- = Absence of allele; 0 = No symptoms (highly resistant); 1 = Slight symptoms visible only on close inspection (resistant); 2 = Intermediate symptoms visible on part of the plant (moderately resistant); 3 = Severe symptoms over the entire plant (moderately susceptible); 4 = Severe symptoms and stunting of the entire plant (susceptible).

<sup>a</sup> TSWV = *Tomato spotted wilt virus*.

<sup>b</sup> ToMV = *Tomato mosaic virus*.

<sup>c</sup> SCAR = Sequence characterized amplified region.

<sup>d</sup> ARMS = Amplification-refractory mutation system.

<sup>e</sup> *rr* = Susceptibility allele, homozygote.

<sup>f</sup> *RR* = Resistance allele, homozygote.

<sup>g</sup> *Rr* = Heterozygote.

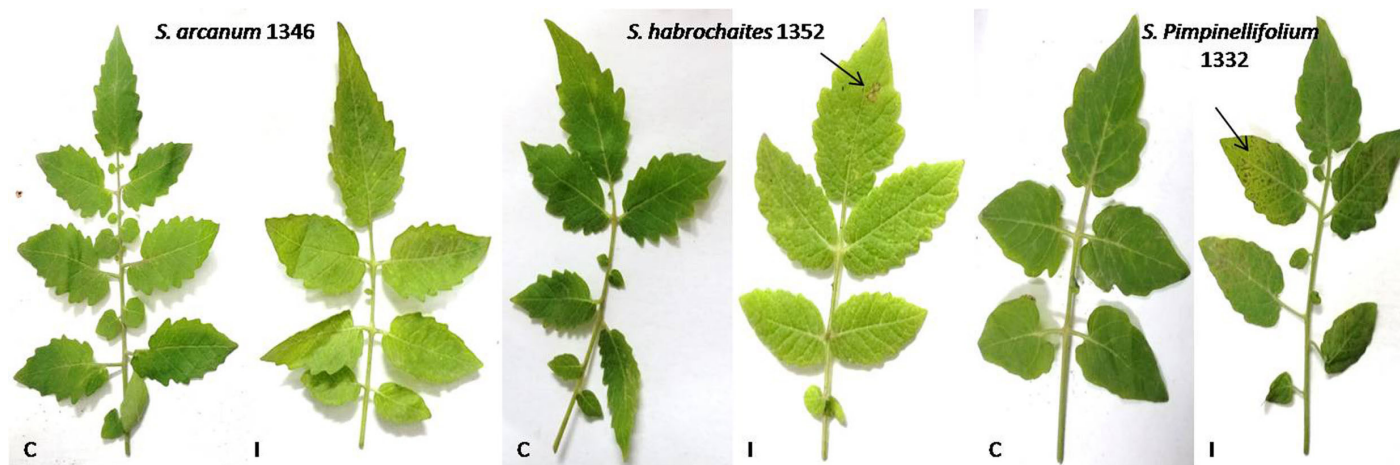
the RedSafe Nucleic Acid Staining Solution (1/20,000) (iNtRON Biotechnology, Inc. Kr), and were visualized with UV light.

### 3. RESULTS

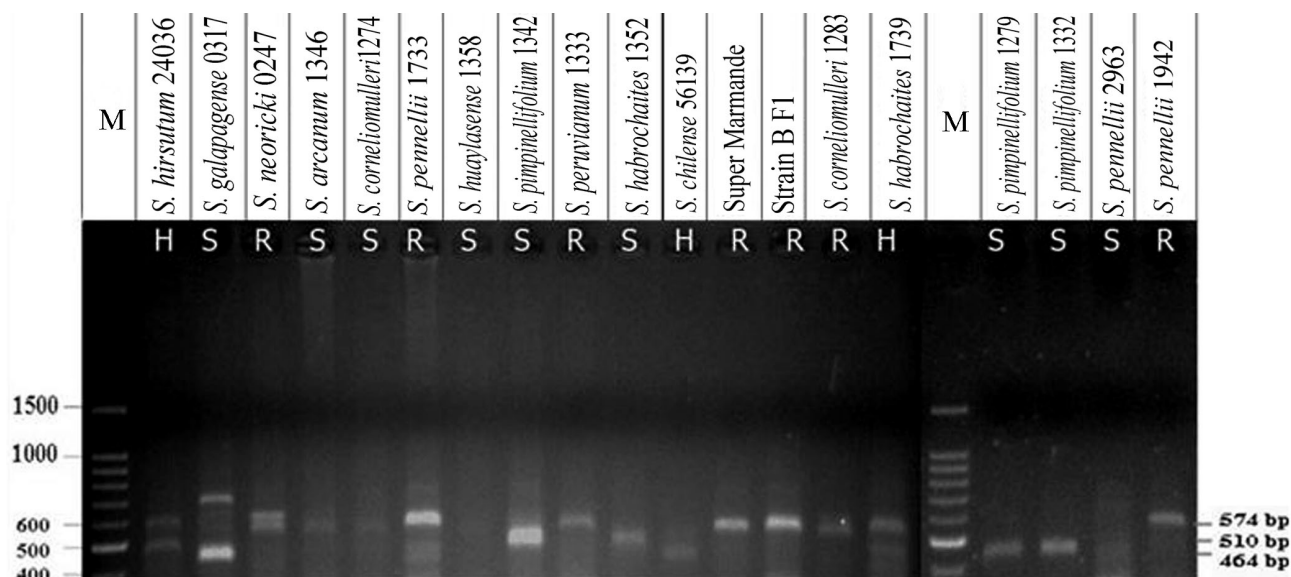
#### 3.1. Characterization of ToMV and TSWV Diseases

Phenotypic characterization of 19 tomato genotypes against the TSWV and ToMV under greenhouse conditions will reflect their field performance. In fact, two tomato genotypes were categorized

as highly resistant to the TSWV, eight resistant, four moderately resistant, four moderately susceptible, and one susceptible to TSWV infection (Fig. 3 and Table 3). For the ToMV, 11 lines were highly resistant; 5 genotypes were resistant, 2 moderately resistant, and 1 moderately susceptible (Fig. 4 and Table 3). The phenotype results were confirmed by TSWV and ToMV ImmunoStrip Kits. Resistance and susceptibility to viral infection were clearly distinguished by the appearance of the colored band.



**Figure 4:** Phenotype of inoculated tomato genotypes three weeks after inoculation with ToMV virus. C: control, I: inoculated plants with ToMV. *S. arcanum* 1346, *S. habrochaites* 1352, and *S. pimpinellifolium* 1332 showing (mild mosaic), (mild mosaic and necrotic spot) and necrotic spot, respectively. The arrows refer to necrotic spots.



**Figure 5:** PCR fragments represent primer set Sw5 SCAR amplified from 19 tomato genotypes, resolved in 1% agarose gel. Lane M: 100 bp DNA ladder; R = homozygous resistant genotypes; S = susceptible genotypes; H = heterozygote resistant genotypes.

### 3.2. Gene-Based Marker for *Sw5* and *Sw5b* Resistance

Two gene-derived SCAR markers (*Sw5* SCAR and *Sw5b* SCAR) (Table 3) were used to detect *Sw5* and *Sw5b* resistance genes, respectively, responsible for resistance to TSWV disease.

#### 3.2.1. *Sw5*

PCR amplification was performed with primer pair Sw5 SCAR, using genomic DNA extracted from 19 tomato lines (Fig. 5). The PCR results indicated four DNA fragments. The first group displayed a single fragment of 574 bp which was scored by nine tomato genotypes carrying the *Sw-5* locus involving *S. corneliomulleri* 1274 and 1283, *S. pennellii* 1733 and 1942, *S. neoricki* 0247, *S. peruvianum* 1333, *S. arcanum* 1346, *S. lycopersicon* cv. *Super Marmande*, and *S. lycopersicon* cv. *Strain B F1*. The second group yielded one

band of 510 bp and included two susceptible genotypes, *S. pimpinellifolium* 1342 and *S. habrochaites* 1352. The third group exhibited one band of 464 bp and consisted of the two susceptible tomato lines, *S. pimpinellifolium* 1279 and 1332. The fourth group gave two amplified fragments of 464 and 574 bp, e.g., *S. hirsutum* 24036, *S. galapagense* 0317, and *S. habrochaites* 1739. Those were heterozygous for the *Sw5* locus. On the other hand, *S. huaylasense* 1358 and *S. pennellii* 2963 did not score any PCR products (Fig. 5).

#### 3.2.2. *Sw5b*

PCR experiments were conducted on DNA isolated from 19 tomato lines by the primer pair Sw5b SCAR. PCR results recorded an amplicon of 541 bp in all tomato genotypes studied except *S. pimpinellifolium* 1332 and *S. hirsutum* 24036, which confer the presence of resistance gene *Sw5b* (Fig. 6).



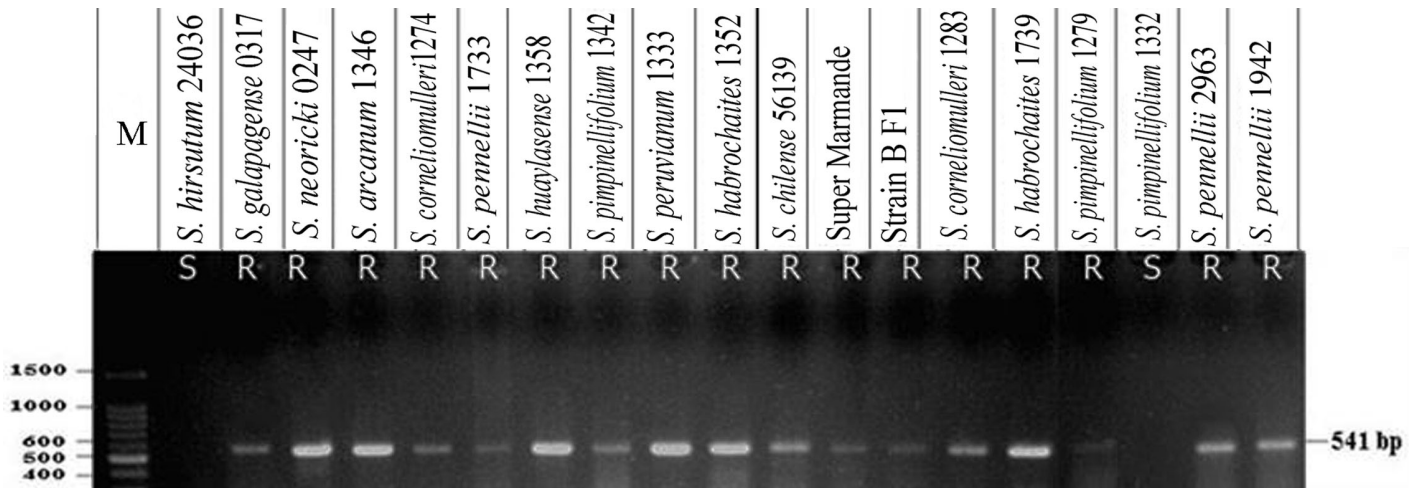


Figure 6: PCR fragments represent primer pair Sw5b SCAR amplified from 19 tomato genotypes, resolved in 1% agarose gel. Lane M = 100 bp DNA ladder; R = homozygous resistant genotypes; S = susceptible genotypes.

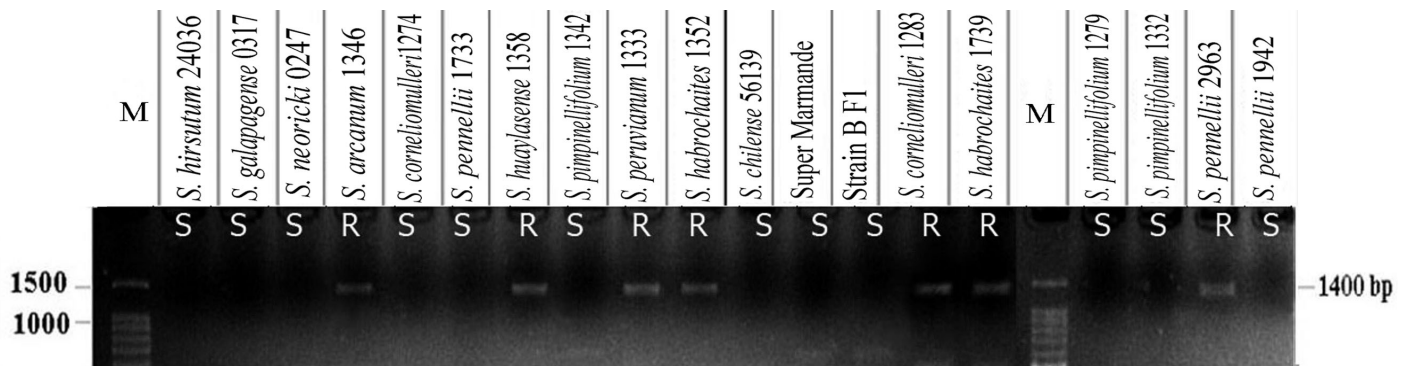


Figure 7: PCR fragments represent primer pair Tm-1SCAR amplified from 19 tomato genotypes, resolved in 1% agarose gel. Lane M: 100 bp DNA ladder; R = homozygous resistant genotypes; S = susceptible genotypes.

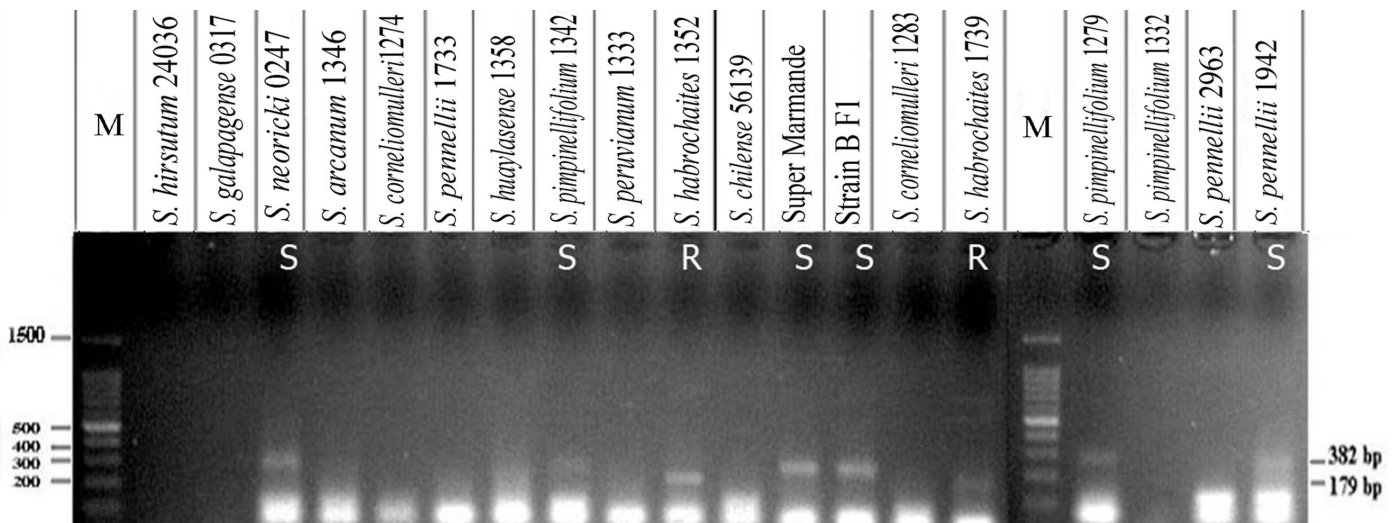


Figure 8: PCR fragments represent primer pair Tm<sup>2</sup> ARMS amplified from 19 tested tomato genotypes, resolved in 1% agarose gel. Lane M: 100 bp DNA ladder, R = homozygous resistant genotypes; S = susceptible genotypes.

### 3.3. Gene-Based Marker for *Tm-1*, *Tm-2*, *Tm-2<sup>2</sup>*, and *tm-2<sup>2</sup>* Resistance Genes

Two gene-tagged markers, *Tm-1* SCAR and *Tm-2<sup>2</sup>* ARMS related to ToMV resistance genes, were used to select tomato genotypes that have resistance genes *Tm-1* and *Tm-2* or *Tm-2<sup>2</sup>*, respectively.

#### 3.3.1. *Tm-1*

The primer pair *Tm-1* SCAR amplified one amplicon of 1,400 bp from seven tomato lines carrying the *Tm-1* resistance allele, e.g., *S. arcanum* 1346, *S. pennellii* 2963, *S. corneliomulleri* 1283, *S. huaylasense* 1358, *S. habrochaites* 1352 and 1739, and *S. peruvianum* 1333 (Fig. 7).

#### 3.3.2. *Tm-2* or *Tm-2<sup>2</sup>* or *tm-2<sup>2</sup>*

A total of 19 tomato lines were exposed to the ARMS-PCR assay. Primer set *Tm-2<sup>2</sup>* ARMS yielded one amplified fragment of 179 bp for the dominant allele *Tm-2* or *Tm-2<sup>2</sup>* in two accessions, *S. habrochaites* 1352 and 1739. Furthermore, the same primer scored one fragment of 382 bp for recessive allele *tm-2<sup>2</sup>* in *S. pimpinellifolium* 1342, *S. neoricki* 0247, *S. lycopersicon* cv. Super Marmande, *S. lycopersicon* cv. Strain B F1, *S. pimpinellifolium* 1279, and *S. pennellii* 1942 (Fig. 8).

## 4. DISCUSSION

For the time being, several dominant and recessive resistance loci to the TSWV (*Sw5b/sw5b* and *Sw5-2/sw5-2*) and ToMV (*Tm-1/tm-1*, *Tm-2/tm-2*, and *Tm-2<sup>2</sup>/tm2<sup>2</sup>*) were identified. The majority of these genes either do not permit or prevent replication of the virus. To detect these genes, four molecular markers (three SCAR and one ARMS) were employed in this study to screen tomato genotypes that have resistance genes for marker-assisted selection (MAS) programs.

In this respect, 19 tomato lines were classified as highly resistant to the TSWV under the glasshouse conditions (*S. peruvianum* 1333 [30] and *S. chilense* 56139 [31]), eight resistant (*S. neoricki* 0247, *S. corneliomulleri* 1274 [32], *S. pennellii* 1733 and 1942, *S. huaylasense* 1358 [32], *S. habrochaites* 1352 and 1739 [1], and *S. lycopersicon* cv. Super Marmande), four moderately resistant (*S. hirsutum* 24036, *S. galapagense* 0317, and *S. pimpinellifolium* 1342 and 1279), and four moderately susceptible (*S. arcanum* 1346 [32], *S. lycopersicon* cv. Strain B, *S. pimpinellifolium* 1332, and *S. pennellii* 2963 [33]). These genotypes have dominant or recessive alleles with homozygous or heterozygous *Sw5* or *Sw5b* or both. These results were confirmed by Gordillo *et al.* [30] who found TSWV resistance in wild tomato genotypes, and some of the resistance loci were introgressed into domesticated tomato cultivars. The *Sw1a* and *Sw1b* loci were quickly overcome by TSWV isolates. However, *Sw-6* and *Sw-7* confer partial resistance to a small range of TSWV isolates, but they are not well identified and not widely applied in domesticated tomato cultivars.

In the current investigation, one tomato accession, *S. pimpinellifolium* 1332, was found to have recessive alleles *sw5* and *sw5b*, indicating that it is moderately susceptible to the TSWV [32]. Although line *S. corneliomulleri* 1283 has dominant alleles with homozygous *Sw5* and *Swb*, it is susceptible to TSWV infection, which is attributed to some TSWV strains being able to overcome *Sw* resistance genes

[30]. These findings were in line with those reported by Pappu *et al.* [34,35]; de Oliveria indicated that mutation in *Sw-5* proteins identified in tomato genotypes susceptible to the TSWV does not recognize the avr protein of the virus. In addition, two amino acids (aa) exchanges in movement proteins (NSm), NSm<sup>C118Y</sup> or NSm<sup>T120N</sup>, overcome *Sw-5b*-mediated resistance by TSWV isolates. A single mutation in the NSs protein, T104A, overcomes *Tsw*-mediated resistance. Aramburu *et al.* [36] indicated that resistance-breaking isolates of the TSWV in Spain are able to overcome the resistance referred to by the *Sw-5* locus in tomatoes.

In this article, we observed that a commercial tomato cultivar ("Super Marmande") gave resistance to the TSWV, which carries both alleles *Sw5* and *Sw-5b*. This result was confirmed by Shi *et al.* [37] who selected 14 tomato genotypes and 10 domesticated tomato genotypes for resistance against the TSWV and indicated that only three domesticated genotypes ("BHN-444," "Sophya," and "Talladega") and one wild species (LA3667) carry the resistance allele *Sw-5b*.

In our study, 11 tomato genotypes were highly resistant to the ToMV, namely, *S. neoricki* 0247, *S. corneliomulleri* 1274 and 1283 [38], *S. pennellii* 1733, 2963, and 1942, *S. huaylasense* 1358 [38], *S. chilense* 56139, *S. lycopersicon* cv. Super Marmande, *S. habrochaites* 1739 [12–13], and *S. pimpinellifolium* 1279 [38], five genotypes were resistant (*S. hirsutum* 24036, *S. galapagense* 0317, *S. arcanum* 1346 [38], *S. peruvianum* 1333 [14,15], and *S. lycopersicon* cv. Strain B), two accessions were moderately resistant (*S. pimpinellifolium* 1342 and 1332 [38]), and one line was moderately susceptible (*S. habrochaites* 1352). These lines have homozygous dominant or recessive alleles *Tm-1* or *Tm-2* or *Tm-2<sup>2</sup>* or *tm2<sup>2</sup>* separately or mixed. However, *S. neoricki* 0247, *S. pimpinellifolium* 1342 and 1279, *S. lycopersicon* cv. Super Marmande, *S. lycopersicon* cv. Strain B, and *S. pennellii* 1942 all have homozygous recessive allele *tm2<sup>2</sup>*, which confers resistance to the ToMV. The resistance to the ToMV may be related to a recessive locus *tm2<sup>2</sup>*, which is controlled by epistatic interactions. These results were synchronized with the results of Diaz-Pendon *et al.* [39] who showed that recessive loci have been related to plant virus resistance and have been ascribed to the plants lacking some basic factors desired for movement or replication of the virus. Consequently, it is possible that resistance to the ToMV may be polygenic and involves both dominant and recessive genes. Hashimoto *et al.* [40] reported that recessive resistance is caused by a mutation in a recessive allele that codes for a host component essential for virus replication. Furthermore, a lack of a negative regulator of defensive responses in the plant host, or the autoactivation of defense signaling, may confer recessive resistance. The most often used recessive resistance loci in diverse crops are eukaryotic translation initiation factor (eIF) 4E and eIF4G, which are effective against a broader range of plant viruses. Mutation in eIF4Es refers to loss of susceptibility to many viruses. It is critical to find new genetic sources for recessive resistance against plant pathogenic viruses in order to develop crops that rely on recessive resistance against a wide range of plant viruses.

In this work, some tomato accessions have no dominant or recessive loci and recorded resistance to the ToMV, e.g., *S. hirsutum* 24036, *S. pennellii* 1733, *S. galapagense* 0317, *S. corneliomulleri* 1274, *S. chilense* 56139, and *S. pimpinellifolium* 1332. This resistance may be due to the presence of new genes in the plant host which



refer to resistance against the ToMV. These results agreed with Rasul's results [38] who isolated six genes homologous to the *Tm2*<sup>2</sup> locus, which depending on their resistance phenotype were named *ScoTm*, *Satm-2*, *Sctm-2*, *ShTm-2*, *SpiTm-2*, and *Sptm-3* from *S. corneliomulleri* LA1292, *S. arcanum* LA2172, *S. chilense* LA 2884, *S. huaylasense* LA1982, *S. pimpinellifolium* LA0722, and *S. peruvianum* LA0752, respectively. Ciuffo *et al.* [41] and Verlaan *et al.* [42] mentioned that several virus resistance loci (dominant or recessive) had been identified and applied in the breeding programs of different crops. The majority of these loci either do not permit or prevent replication of the virus into the plant cells.

In the present work, all studied tomato genotypes were resistant against both the TSWV and ToMV except *S. arcanum* 1346, *S. lycopersicon* cv. Strain B, *S. corneliomulleri* 1283, *S. pimpinellifolium* 1332, and *S. pennellii* 2963 as well as *S. habrochaites* 1352, which were moderately susceptible and susceptible to the TSWV and ToMV, respectively.

## 5. CONCLUSION

Gene-based markers screening for the detection of pathogen resistance genes in different tomato genotypes has become a valuable tool in plant viruses resistance. In this paper, we have applied DNA markers to detect resistance loci to the TSWV and ToMV in 19 tomato genotypes. In this investigation, we have identified 18 tomato genotypes bearing the dominant allele for TSWV resistance. In addition, seven lines have resistance genes to the ToMV. Therefore, the newness of this research is the identification of donor parents for producing tomato genotypes resistant to both the TSWV and ToMV in tomato breeding programs or the production of tomato lines with pyramided genes for resistance to several viruses through MAS.

## 6. CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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## 8. CONSENT TO PARTICIPATE

Not applicable.

## 9. ETHICAL APPROVAL

Not applicable.

## 10. AUTHORS' CONTRIBUTIONS

Dr. HAM carried out SCAR and ARMS markers and data analysis and interpretation, Prof. Dr. SAM performed virus resistance tests and writing of the manuscript, and MEO corrected and edited the manuscript.

## 11. DATA AVAILABILITY

All data generated or analyzed during this investigation already exist in this paper.

## 12. LOCAL AND NATIONAL REGULATIONS

All studies follow local and national regulations.

## REFERENCES

1. Saidi M, Warade SD. Tomato breeding for resistance to *Tomato spotted wilt virus* (TSWV): an overview of conventional and molecular approaches. *Czech J Genet Plant Breed* 2008;44:83–92.
2. FAOSTAT. Statistics Division of the Food and Agricultural Organization of the United Nations. 2019. Available via <https://www.fao.org/faostat/en/#data/QC>
3. Kang BC, Yeom I, Jahn MM. Genetics of plant virus resistance. *Annu Rev Phytopathol* 2005;43:581–621.
4. German TL, Ullman DE, Moyer JW. *Tospoviruses*: diagnosis, molecular biology, phylogeny, and vector relationships. *Annu Rev Phytopathol* 1992;30:315–48.
5. Pappu HR, Jones RAC, Jain RK. Global status of *Tospovirus* epidemics in diverse cropping systems: successes achieved and challenges ahead. *Virus Res* 2009;141:219–36.
6. Finlay K. Inheritance of spotted wilt resistance in the tomato II. Five genes controlling spotted wilt resistance in four tomato types. *Aust J Biol Sci* 1953;6:153–63.
7. Price DL, Memmott FD, Scott JW, Olson SM, Stevens MR. Identification of molecular markers linked to a new *Tomato spotted wilt virus* resistance source in tomato. *Tomato Genet Coop* 2007;57:35–6.
8. Spassova MI, Prins TW, Folkertsma RT, Klein-Lankhorst RM, Hille J, Goldbach RW, *et al.* The tomato gene *Sw5* is a member of the coiled coil, nucleotide binding, leucine-rich repeat class of plant resistance genes and confers resistance to TSWV in tobacco. *Mol Breed* 2001;7:151–61.
9. Van Zijl J, Bosch S, Coetzee C. Breeding tomatoes for processing in South Africa. In: Strydom DK (ed.). *International symposium on fruit & vegetables for processing 194*, Acta Horticulturae, Cape Town, South Africa, pp 69–76, 1985.
10. Stevens M, Scott S, Gergerich R. Inheritance of a gene for resistance to *Tomato spotted wilt virus* (TSWV) from *Lycopersicon peruvianum* Mill. *Euphytica* 1992;59:9–17.
11. Boiteux L, Giordano LdB. Genetic basis of resistance against two *Tospovirus* species in tomato (*Lycopersicon esculentum*). *Euphytica* 1993;71:151–4.
12. Pelham J. Resistance in tomato to *Tobacco mosaic virus*. *Euphytica* 1966;15:258–67.
13. Watanabe Y, Kishibayashi N, Motoyoshi F, Okada Y. Characterization of *Tm-1* gene action on replication of common isolates and a resistance-breaking isolate of TMV. *Virology* 1987;161:527–32.
14. Nishiguchi M, Motoyoshi F. Resistance mechanisms of *Tobacco mosaic virus* strains in tomato and tobacco. In: Evered D, Harnett S (ed.). *Plant resistance to viruses*, John Wiley & Sons, Chichester, UK, pp 38–46, 1987.
15. Meshi T, Motoyoshi F, Maeda T, Yoshikawa S, Watanabe H, Okada Y. Mutations in the *Tobacco mosaic virus* 30-kDa protein gene overcome *Tm-2* resistance in tomato. *Plant Cell* 1989;1:515–22.
16. Cirulli M, Alexander LJ. Influence of temperature and strain of *Tobacco mosaic virus* on resistance in a tomato breeding line derived from *Lycopersicon peruvianum*. *Phytopathology* 1969;59:1287–97.
17. Hall TJ. Resistance at the *Tm-2* locus in the tomato to *Tomato mosaic virus*. *Euphytica* 1980;29:189–97.
18. Young ND, Tanksley SD. RFLP analysis of the size of chromosomal segments retained around the *Tm-2* locus of tomato during backcross breeding. *Theor Appl Genet* 1989;77:353–9.
19. Fraser RSS. The genetics of resistance to plant viruses. *Annu Rev Phytopathol* 1990;28:179–200.
20. Lanfermeijer FC, Dijkhuis J, Sturre MJ, de Haan P, Hille J. Cloning and characterization of the durable *Tomato mosaic virus* resistance gene *Tm-2(2)* from *Lycopersicon esculentum*. *Plant Mol Biol* 2003;52:1037–49.

21. Lanfermeijer FC, Warmink J, Hille J. The products of the broken *Tm-2* and the durable *Tm-2(2)* resistance genes from tomato differ in four amino acids. *J Exp Bot* 2005;56:2925–33.
22. Dinh QD, Dechesne A, Furrer H, Taylor G, Visser RGF, Harbinson J, *et al.* High-altitude wild species *Solanum arcanum* LA385-A potential source for improvement of plant growth and photosynthetic performance at suboptimal temperatures. *Front Plant Sci* 2019;10:1163; doi:10.3389/fpls.2019.01163
23. Mahfouze SA, Mahfouze HA. Comparison between CAPS and SCAR markers for detection of *Tomato yellow leaf curl virus* and whitefly resistance genes in tomato genotypes. *Jordan J Biol Sci* 2019;12(2):123–33.
24. Green SK. Guidelines for diagnostic work in plant virology. 2nd edition, Asian Vegetable Research and Development Center, Taipei, Taiwan, p 63, 1991.
25. Hutton SF, Scott JW. *Ty-6*, a major begomovirus resistance gene located on chromosome 10. *Rep Tomato Genet Coop* 2014;64:14–8.
26. Dianese E'C, de Fonseca MEN, Goldbach R, Kormelink R, Inoue-Nagata AK, Resende RO, *et al.* Development of a locus-specific, co-dominant SCAR marker for assisted-selection of the *Sw-5* (*Tospovirus* resistance) gene cluster in a wide range of tomato accessions. *Mol Breed* 2010;25:133–42.
27. Shi A, Vierling R, Grazzini R, Chen P, Caton H, Panthee D. Identification of molecular markers for gene of *Sw-5* *Tomato spotted wilt virus* resistance. *Am J Biotechnol Mol Sci* 2011a;1:8–16.
28. Ohmori T, Murata M, Motoyoshi F. Molecular characterization of RAPD and SCAR markers linked to the *Tm-1* locus in tomato. *Theor Appl Genet* 1996;92:151–6.
29. Arens P, Mansilla C, Deinum D, Cavellini L, Moretti A, Rolland S, *et al.* Development and evaluation of robust molecular markers linked to disease resistance in tomato for distinctness, uniformity and stability testing. *Theor Appl Genet* 2010;120(3):655–64.
30. Gordillo LF, Stevens MR, Millard MA, Geary B. Screening two *Lycopersicon peruvianum* collections for resistance to *Tomato spotted wilt virus*. *Plant Dis* 2008;92:694–704.
31. Stevens MR, Scott SJ, Gergerich RC. Evaluation of seven *Lycopersicon* species for resistance to *Tomato spotted wilt virus* (TVSW). *Euphytica* 1994;80:79–84.
32. Li J, Huang H, Zhu M, Huang S, Zhang W, Dinesh-Kumar SP, *et al.* A plant immune receptor adopts a two-step recognition mechanism to enhance viral effector perception. *Mol Plant* 2019;12:248–62.
33. Stevens MR, Lamb EM, Rhoads DD. Mapping the *Sw5* locus for *Tomato spotted wilt virus* resistance in tomatoes using RAPD and RFLP analyses. *Theor Appl Genet* 1995;90:451–6.
34. Pappu HR, Anna E Whitfield, de Oliveira AS. *Tomato spotted wilt virus* (*Tospoviridae*). *Encycl Virol* 2008;133–8.
35. de Oliveira AS, Boiteux LS, Kormelink R, Resende RO. The *Sw-5* gene cluster: tomato breeding and research toward Orthospovirus disease control. *Front Plant Sci* 2018;9:1055; doi:10.3389/fpls.2018.01055.
36. Aramburu J, Galipienso L, Soler S, López C. Characterization of *Tomato spotted wilt virus* isolates that overcome the *Sw-5* resistance gene in tomato and fitness assays. *Phytopathol Mediterr* 2010;49:342–51.
37. Shi A, Vierling R, Grazzini R, Chen P, Caton H, Panthee D. Molecular markers for *Tm-2* alleles of *Tomato mosaic virus* resistance in tomato. *Am J Plant Sci*. 2011b;2:180–9.
38. Rasul I. TMV resistance in different *Solanum* species. In: Lanfermeijer FC, Hille J (ed.). Characterization of the *Tm-2* locus of tomato and its durability, University of Groningen, Groningen, Netherlands, pp 107–28, 2012.
39. Diaz-Pendon J, Truniger V, Nieto C, Garcia-Mas J, Bendahmane A, Aranda M. Advances in understanding recessive resistance to plant viruses. *Mol Plant Pathol* 2004;5:223–33.
40. Hashimoto M, Neriya Y, Yamaji Y, Namba S. Recessive resistance to plant viruses: potential resistance genes beyond translation initiation factors. *Front Microbiol* 2016;7:1695; doi:10.3389/fmicb.2016.01695
41. Ciuffò MC, Finetti-Sialer MM, Gallitelli D, Turina M. First report in Italy of a resistance breaking strain of *Tomato spotted wilt virus* infecting tomato cultivars carrying the *Sw5* resistance gene. *N Dis Rep* 2005;10:48.
42. Verlaan MG, Szinay D, Hutton SF, de Jong H, Kormelink R. Chromosomal rearrangements between tomato and *Solanum chilense* hamper mapping and breeding of the TYLCV resistance gene *Ty-1*. *Plant J* 2011;68:1093–103.

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