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Genetic control of two novel sources of tomato brown rugose fruit virus resistance in *Solanum pimpinellifolium*

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Abstract

Two new sources of high resistance to tomato brown rugose fruit virus (ToBRFV) were identified in Solanum pimpinellifolium with unknown genetic control and location. The two wild tomato lines were each crossed to a susceptible inbred line to create two genetic mapping populations. A total of 166 and 162 individuals from each population were phenotyped for resistance to ToBRFV. The segregation pattern for each population suggested resistance was controlled by two recessive genes. The genetic location of the resistance loci is currently being mapped in both populations.

Introduction

Tomato brown rugose fruit virus (ToBRFV) is the major pathogen that threatens greenhouse tomato production due to its high transmissibility and ability to cause severe foliar and fruit symptoms. Identifying resistant tomato germplasm is an important step toward developing ToBRFV-resistant cultivars. Two *S. pimpinellifolium* lines with high resistance to ToBRFV were recently identified; however, the genetic basis and chromosomal locations of their resistance were unknown.

The objective of this study was to investigate the genetic control of these two sources of ToBRFV resistance, by crossing each resistant line to a susceptible inbred tomato line to generate two F2 populations. To confirm the segregation pattern of the resistance, individuals from each population were phenotyped for the response to ToBRFV infection based on disease severity score (DSS).

Methodology

Preparation of plant material

Two F2 populations, consisting of 166 and 162 individuals, respectively, were developed by crossing each resistant tomato line to a susceptible inbred line. Seeds were sown in rockwool propagation
sheets and cuttings were taken from individual plants to create 2 replicates for phenotyping. Propagated seedlings were transplanted into 6-inch pots prior to moving from a greenhouse into an advanced pathology lab.

Inoculation of plants

The second and third true leaves were mechanically inoculated with a Canadian isolate of ToBRFV. The second and third true leaves were lightly dusted with carborundum powder and rubbed with a sterile cotton swab dipped in viral inoculum, prepared by grinding infected tomato tissue with 0.01 M sodium phosphate buffer (pH 7.0) in a 1:10 w/v ratio. Disease phenotype was visually assessed using an adapted version of the disease severity index developed by Jewehan et al., 2022. Symptoms were assessed 20 days post inoculation. Following this, 0.3 g of the inoculated leaf tissue was collected and tested using the Agdia® ToBRFV Double Antibody Sandwich (DAS) Enzyme-Linked Immunosorbent Assay (ELISA) kit, following the manufacturer's instructions.

Table 1. Disease severity score on plants inoculated with ToBRFV

Disease Severity Score	Symptoms
0	No Symptoms
1	Mild Mosaic or Mottling
2	Mosaic
3	Mosaic and/or Leaf Deformations
4	Mosaic and Leaf Deformations, Shoestring
5	Severe Mosaic & Leaf Deformations, Shoestring, Rolling, Stunting

Adapted from Jewehan et al., 2022

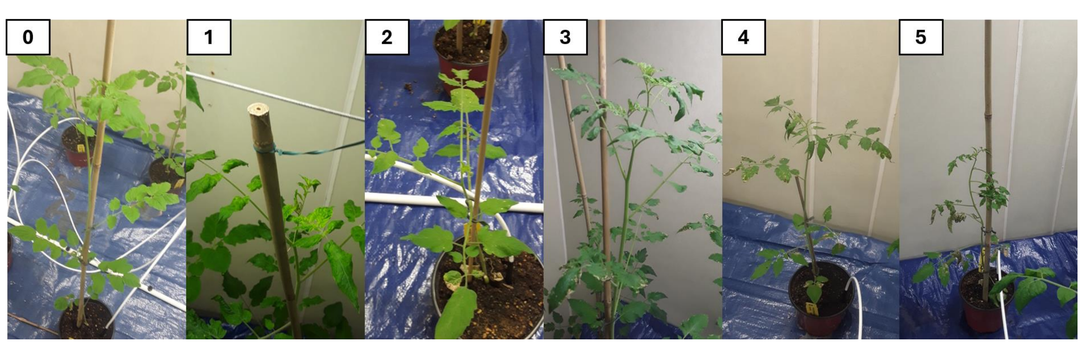

Figure 1. Disease Severity Score and Symptoms of ToBRFV in Vineland's tomato mapping populations.

0: Disease Severity Score of 0 – Plant is healthy.
1: Disease Severity Score of 1 – Mild mottling on limited leaves
2: Disease Severity Score of 2 – Mottling on multiple leaves
3: Disease Severity Score of 3 - Mottling on multiple leaves and deformed leaves
4: Disease Severity Score of 4 – Mottling on multiple leaves, deformed leaves and early shoestring
5: Disease Severity Score of 5 – Mosaic and mottling on multiple leaves. Deformed apical leaves with dead lower leaves. Overall, the plant has experienced stunted growth.

Results

Phenotypic screening of two independent F2 populations was performed to evaluate resistance against a virulent Canadian isolate of ToBRFV. Disease severity was evaluated 20 days post-inoculation (DPI) using a disease severity score scale of 0 to 5. Both F2 populations revealed a skewed distribution heavily favoring disease susceptibility, with fully resistant individuals making up just 4.9% of Population1 and 8.0% of Population 2 (Figure 2).

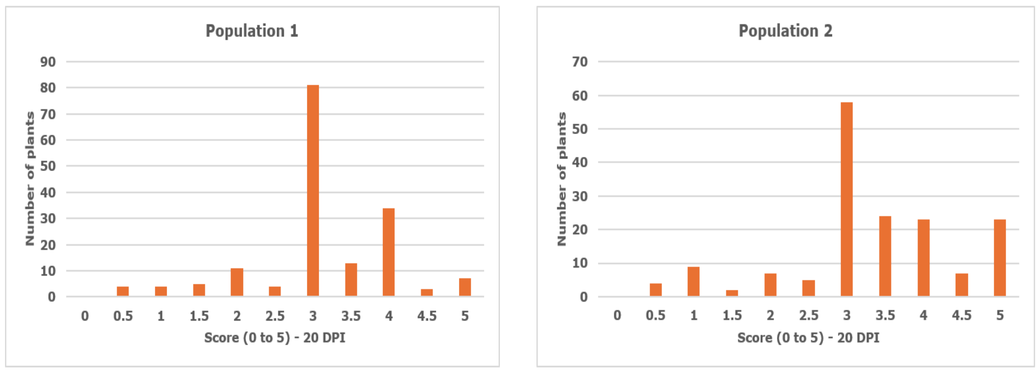

Figure 2. Frequency distribution of two F2 populations for ToBRFV disease severity score (DSS) 20 days post inoculation (DPI)

Plants exhibiting no or minimal viral symptoms (scores 0.0–1.0) were categorized as resistant, whereas individuals displaying distinct mottling or mosaic on multiple leaves, and deformed leaves (scores 1.5–5.0) were categorized as susceptible. Chi-Square (X^2) goodness-of-fit test verified that the phenotypic distribution in both populations fits a 1:15 (Resistant : Susceptible) dihybrid inheritance model (Table 2).

Table 2. Chi-square goodness-of-fit analysis for ToBRFV phenotypic segregation in two F2 populations.

Population	Phenotype	Observed (O)	Expected (E)*	(O-E)	$(O-E)^2 / E$	Chi-Square total	p-value	Fit Assessment
Population 1	Resistant	8	10.38	-2.38	0.544	**0.58**	~0.45	**Fits 1:15 gene model**
	Susceptible	158	155.63	2.38	0.036			
Population 2	Resistant	13	10.13	2.88	0.816	**0.87**	~0.35	**Fits 1:15 gene model**
	Susceptible	149	151.88	-2.88	0.054			

The 1:15 distribution indicates a **duplicate dominant epistasis** model. ToBRFV resistance in these two sources of wild tomato accessions behave as a double homozygous recessive trait (aabb), occurring when both independent susceptibility loci lack a functional dominant allele (Figure 3).

	(AB) (1/4)	(Ab) (1/4)	(aB) (1/4)	(ab) (1/4)
(AB) (1/4)	(AABB) Susceptible	(AABb) Susceptible	(AaBB) Susceptible	(AaBb) Susceptible
(Ab) (1/4)	(AABb) Susceptible	(AAbb) Susceptible	(AaBb) Susceptible	(Aabb) Susceptible
(aB) (1/4)	(AaBB) Susceptible	(AaBb) Susceptible	(aaBB) Susceptible	(aaBb) Susceptible
(ab) (1/4)	(AaBb) Susceptible	(Aabb) Susceptible	(aaBb) Susceptible	(aabb) Resistant

- **Phenotypic Ratio Summary: 1 Resistant ( ) : 15 Susceptible ( )**

Figure 3. Dihybrid Punnett square depicting a 1:15 duplicate dominant epistasis model for ToBRFV resistance.

Discussion

Phenotypic screening of two independent F2 populations demonstrates an exact fit to a 1:15 dihybrid segregation ratio, confirming that ToBRFV resistance is governed by a duplicate dominant epistasis model. Under this model, the two new sources of resistance are controlled by a double homozygous recessive trait (aabb) occurring at a low frequency in the F2 generation. Because visual screening can misidentify heterozygous individuals, breeding programs must utilize high-throughput marker-assisted selection (MAS) to target the (aabb) genotype. Furthermore, to produce completely resistant commercial F2 hybrids, both parental lines must be fixed for the double homozygous recessive state. The data from this study will be used to genetically map this new source of resistance and to develop genetic markers for introgression into elite tomato parental lines.

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