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Sm gene confers tolerance to ToBRFV in tomato

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ABSTRACT

Tomato brown rugose fruit virus (ToBRFV) infection significantly reduces plant vigor, fruit size, and quality, and in the long term affects international seed trade. Several genes have been reported to restrict the systemic spread of ToBRFV in tomato, thereby conferring partial tolerance. We hypothesized that the *SmRR* gene, an incomplete dominant resistant gene against *Stemphylium* sp. in tomato, may also contribute to tolerance against ToBRFV infection. To test this, tomato varieties containing the *SmRR* or *Smrr* genes were mechanically inoculated with ToBRFV. Symptom severity on leaves was recorded at one, two, and three weeks post-inoculation using a standardized disease rating scale, and viral presence was confirmed by RT-PCR. Results showed that the *Smrr* (susceptible) variety exhibited the highest disease severity (maximum score= 3), while the *SmRR* variety had a lower average severity score (1.5) and no visible fruit symptoms, indicating tolerance. Future studies should employ functional assays, such as gene knockdown or overexpression, to validate the role of *SmRR* in ToBRFV tolerance.

1. Introduction

The cultivation of tomato (*Solanum lycopersicum*) is an economically important vegetable but its cultivation and yield potential is threatened by an array of biotic and abiotic stresses. Among biotic stresses viruses in particular Tobomoviruses, threaten their yield and fruit quality.

Members of this family exhibit high stability and can survive on farm tools and equipment for a long time. Additionally, they are transmitted effectively through mechanical means. Previously, all Tobamoviruses of tomato were effectively controlled by *Tm-1* and *Tm-2* resistant genes (Kubota et al. 2024). However, this resistance was rendered inadequate and ineffective after the emergence of another virulent Tobamovirus (Tomato brown rugose fruit virus-ToBRFV) initially reported in Jordan close to a decade ago (Salem et al. 2016). Subsequently, the presence of the virus has been reported in Europe, Asia, and other locations.

ToBRFV has a single-stranded positive-sense RNA genome which is approximately 6.4 kb in size. The genome generally consists of four open reading frames (ORF), has two subunits of RNA-dependent RNA polymerase (RdRp) complex, a movement protein (MP), and a coat protein (CP) (Salem et al. 2016; Yilmaz and Batuman 2023).

The severity of ToBRFV symptoms is influenced by multiple factors, including environmental conditions, viral titer, tomato cultivar, and plant developmental stage. Typical symptoms of the virus infection on tomato are similar to that of other Tobamovirus infection-mosaic, mottling, and fruit discoloration (Salem et al. 2023; Zinger et al. 2021).

Fungal diseases are major problems in tomato cultivation. These diseases may lead to total yield losses, reduction in yield or distortion of plant growth, especially the Grey leaf spot caused by *Stemphylium* sp. which spreads effectively under warm temperatures and high humidity. Reportedly, this disease does not directly affect tomato fruits but damages leaves & fruit sizeas a result of reduced photosynthetic efficiency. The disease is caused by several species of *Stemphylium*- S. *solani* Weber, S. *floridanum* Hanon and Weber, S.*botryosum* Wallr., and S.*vesicarum* (Wallr.) Simmons (Bashi et al. 1973). Fine mapping identified an incomplete dominant gene, *Sm*, conferring resistance to *Stemphylium* spp. (Su et al. 2019).

Various genes have been reported to confirm some degree of protection to plants against plant viruses and other biotic and abiotic stresses. A critical example is the 'N' resistance gene in *Nicotiana glutinosa* (confers resistance to Tobacco mosaic virus and other tobamoviruses in tobacco*)*, where the gene confers N-mediated resistance to the virus in tomato at 22ºC. However, this resistance is ineffective at elevated temperatures (30ºC) (Zhou et al. 2025). Additionally, *SlRbohB* is reported to be responsible for the resistance against *Botrytis cinerea* and tolerance to drought stress (Li et al. 2015). Also, *SlNLC-1*, which plays a role in tomato's resistance against TYLCV, was also confirmed to be responsible for the crop's resistance to *Stemphylium lycopersici* (Cui et al. 2018). However, the potential cross-protection between Sm-mediated fungal resistance and ToBRFV tolerance has not been previously explored. In this study, to determine the role of the gene in suppressing ToBRFV symptom, mechanic inoculation, symptom scoring and RT-PCR tests were conducted

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to analyze the phenotypic and viral expression level in tomato leaf tissues.

2. Materials and Methods

2.1. Inoculum source

Plant sap containing ToBRFV-Ant-Tom (MT107885, NCBI) was used for mechanical inoculation of ten susceptible tomato plants from each group (through the soft sponge method) (Kabas et al. 2022). Two weeks post-inoculation, symptomatic leaves were tested for the presence of ToBRFV. The virus was maintained on a susceptible variety and subsequently used as the inoculum source for other experiments.

2.2. Plant material, growth conditions

Stemphylium sp. resistant and susceptible tomato seedlings were supplied by Anamas seed company in Antalya, Türkiye. Two-week-old seedlings at the 1–2 true-leaf stage were transplanted into plastic pots containing peat and maintained in a greenhouse at 25 °C with a 16 h photoperiod. Occasionally, the position of the pots was changed to mitigate environmental biases.

2.3. DNA, RNA extraction and quality assessment

Symptom severity was evaluated at weekly intervals for three consecutive weeks on ten plants per treatment group, following the rating scale described by Kabas et al. (2022). Fresh leaf samples showing rugose, mottling and mosaic symptoms from five plants per treatment group, were collected and ground in liquid nitrogen. Total RNA was extracted using the Promega SV Total RNA Isolation Kit (Cat. #Z3100), following the manufacturer's protocol. Additionally, DNA extraction was conducted using a method previously described by (Doyle 1991). RNA concentration and purity were assessed with a Qubit 4 Fluorometer, and integrity confirmed by electrophoresis on 1.5% agarose gel.

2.4. Reverse transcription PCR (RT-PCR) and PCR amplification

Equal amounts of RNA (100 ng) were used for amplification with the GRISP One-Step RT-PCR kit (#GK64.0100). Each 50 µl reaction contained 12.5 µl Fast PCR Master Mix, 1.0 µl forward primer, 1.0 µl reverse primer (2), 2 µl RNA template, 1.25 µl RTase mix, and RNase-free water. PCR cycling conditions included: 45°C for 15 min, 95°C for 3 min, followed by 35 cycles of 95°C for 10 s, 59°C for 10 s, 72°C for 15 s, and a final extension at 72°C for 1 min. The primers used in RT-PCR were adopted from (Cayak and Fidan 2024). PCR products (351 bp) were visualized on a 1.5% agarose gel. Also, for the detection and confirmation of *Smrr* and *SmRR* gene in the tomato variety, a PCR with Sm-InDel (F: CTACAC TTTCTCGTTCCCAATG R: R: 5'-ATCGCCAAACCAATCAAATC-3) primers was used as currently reported by Su et al. (2019). Using the Ecotech PCR mastermix protocol, the PCR was conducted as follows: 98°C for 30 sec followed by 35 cycles of 94°C for 10 s, 55°C for 15 s, 72°C for 15 s, and a final extension at 72°C for 1 min.

2.5. Statistical analysis and data visualization

Scores for ToBRFV symptoms were analyzed using a violin plot with overlaid mean ± standard error (SE).

3. Results

3.1. ToBRFV symptoms on Stemphylium sp. susceptible varieties more than Stemphylium sp. resistant varieties

One, two, and three weeks after the final inoculation, the severity of ToBRFV symptoms on the leaves of the plants was assessed using the disease severity scale described by Kabas et al. (2022). In general, mosaic, rugose, and leaf deformation symptoms were more pronounced in varieties carrying the *Smrr* gene one week after inoculation. A disease severity score of 1 was recorded during the first week (Fig 1), with symptom intensity progressively increasing during the second- and third-weeks post-inoculation. At the third week post-inoculation, typical and archetype symptoms of the virus were dominant on their leaves. As such, most of the plants were scored 3. Contrastly, on the variety that contained the dominant gene, there were no visible symptoms on the leaf a week post-inoculation. Symptoms on the leaf became apparent at second- and third-week post-inoculation. It is worth noting that no symptoms were observed on the ripe fruits of *Stemphylium* sp. resistant variety. (Fig 2 and 3). Phenotypically, a reduction in fruit size was observed in the Smrr-infected variety. The noticeable decrease in fruit size is likely attributable to stress induced by viral infection. Viral infections can impair the plant's nutrient acquisition and translocation processes, thereby negatively influencing its phenotypic traits

3.2. Confirmation of Smrr and SmRR gene and ToBRFV infection in the candidate varieties

The allelic condition of the *Smrr* and *SmRR* loci in the two tomato varieties was first analyzed through the markers in method 2.4. Using the marker, the *Sm* homozygous resistant gene (*SmRR*) generated a band size of approximately 122bp whilst that of the susceptible generated a band size of approximately 140bp (*Smrr*) (Fig 4a).

Also, RT-PCR confirmed ToBRFV infection in both *Stemphylium* sp.- resistant and susceptible plants after RT-PCR tests (Fig 4b).

3. Discussion

In recent years, there have been increasing reports on the rapid spread of Tomato brown rugose fruit virus (ToBRFV) globally (Salem et al. 2022; Salem et al. 2023).The disease causes various symptoms, including color distortion in ripe fruits and leaf deformation (Yilmaz and Batuman 2023). In the present study, tomato varieties carrying the incompletely dominant resistance gene against *Stemphylium* sp. (*SmRR*) exhibited reduced symptom severity on leaves and an absence of fruit symptoms following ToBRFV infection. To further investigate this observation, tomato varieties containing either the susceptible (*Smrr*) or resistant (*SmRR*) gene were mechanically inoculated with ToBRFV. Consistent with field previous field observations, varieties possessing the susceptible gene (*Smrr*) displayed typical viral symptoms, such as mosaic and leaf deformation, within two weeks and three weeks after inoculation. In contrast, visible symptoms appeared later, approximately two weeks post-inoculation, in the *SmRR* genotypes, indicating delayed symptom expression and potential partial tolerance.

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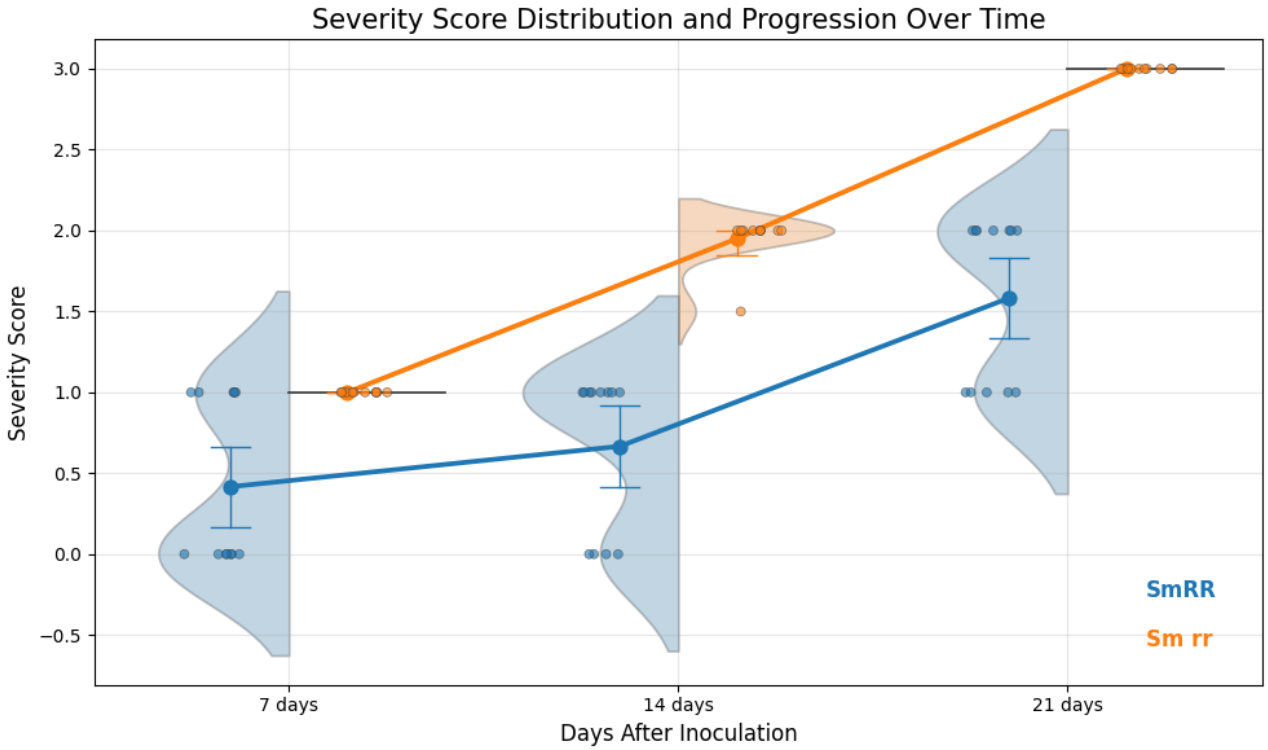


Figure 1. Symptom severity scoring of ToBRFV on leaves of *Stemphylium* sp.-resistant and *Stemphylium* sp.-susceptible varieties at a week, two weeks and three weeks post-inoculation.



Figure 2. ToBRFV symptoms expressed on *Stemphylium* sp. resistant (*SmRR*) and susceptible (*Smrr*) varieties.

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Figure 3. Symptoms of ToBRFV on a) *Stemphylium sp*. resistant variety (mock); b) *Stemphylium* sp. resistant variety (ToBRFV-infected); c) *Stemphylium* sp. susceptible variety (mock); and d) *Stemphylium* sp. susceptible variety (ToBRFV-infected).

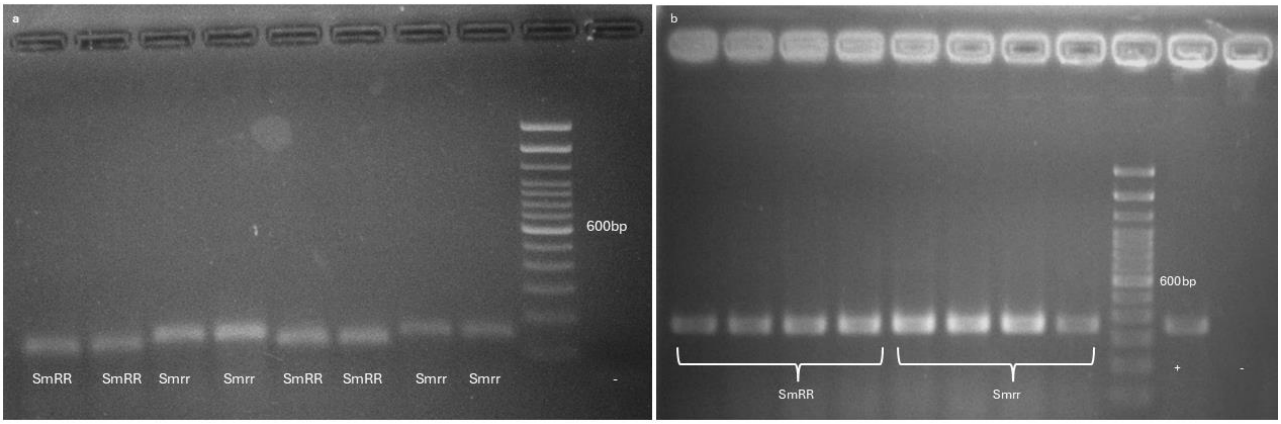


Figure 4. PCR results of a) detection of *Smrr* and *SmRR* gene in tomato varieties; b) RT-PCR detection of ToBRFV infection.

It is well established that some gene loci in crops can exhibit pleiotropic effects, influence multiple traits or provide cross-tolerance to distinct pathogens. Previous studies have reported genomic regions associated with tolerance to ToBRFV. In *Solanum lycopersicum* (tomato) and *S. pennellii* (a wild relative of tomato), a region on chromosome 2 near the *Tm-1* locus has been identified to control resistance and tolerance to ToBRFV (Rochsar et al. 2025; Zinger et al. 2021). It was proposed that at least two other resistance genes may contribute to this tolerance, and additional genomic regions could play a role in modulating viral spread. It is also reported that *Tm-1* gene in addition to QTL2 on chromosome 9 and QTL 3 on chromosome 11 guarantees the complete resistance to the virus (Ashkenazi et al. 2020). The *SmRR* gene (an incomplete dominant gene), located on chromosome 12 (Su et al. 2019), may represent another locus associated with ToBRFV tolerance. The observed reduction in symptom severity in *SmRR*-containing varieties suggests that this gene, directly or through linked resistance pathways, might limit systemic virus movement or trigger host defense responses.

4. Conclusion

In conclusion, tomato varieties that are resistant to *Stemphylium* sp. exhibited some partial tolerance to ToBRFV infection as compared to the susceptible variety. Furthermore, we recommend detailed molecular studies to understand the interaction between the *SmRR* gene and ToBRFV infection.

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