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A comprehensive study on the molecular characterization of tomato spotted wilt orthotospovirus isolates and resistance genes in pepper and tomato

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Abstract: Tomato spotted wilt orthotospovirus (TSWV, *Orthotospovirus tomatomaculae*) resistance genes *(Tsw, Sw5)* have been identified in pepper and tomato plants. The development of resistant cultivars is one of the popular management strategies for overcoming viral infections. However, the breakdown of resistance genes has been documented from many places where resistant cultivars have been developed. This has mainly been due to the emergence of resistance-breaking (RB) lines because of the consecutive use of these cultivars. The development of these isolates may cause great losses in both tomato and pepper plants in Türkiye. To investigate the incidence of TSWV, 150 symptomatic samples were collected from widespread locations of tomato and pepper growing provinces in Türkiye. Samples were inoculated to enhance the virus titer and further confirmed by enzyme-linked immunosorbent assay (ELISA). The ELISA positive samples were confirmed by reverse transcription-polymerase chain reaction. The sequence-characterized amplified region (SCAR) and CAPS markers were used to screen resistance genes in tomato and pepper plants. Positive TSWV-isolates were partially sequenced to determine whether they had the previously reported mutations in *NSs* or *NSm* genes for RB isolates. As per the virus characterization, *NSm* and *NSs* amino acid sequences showed no previously identified mutations. Resistance gene-carrying isolates were amplified for partial genome sequences. Phylogenetic analyses revealed that both *NSs* and *NSm* were distributed in the same genetic pool. The *N* gene sequence comparison and phylogenetic analysis showed that the Turkish isolates were clustered in a separate clade. These findings provide insight into TSWV infections and resistance genes of pepper and tomato by phylogenetic analysis.

Key words: Tomato, pepper, resistance, molecular marker, movement protein, silencing suppressor protein

1. Introduction

Tomato spotted wilt orthotospovirus (TSWV, *Orthotospovirus tomatomaculae*) is a member of the *Bunyavirales* and *Tospoviridae* family (Nilon et al., 2021). *Fimoviridae* and *Tospoviridae* are the 2 crucial plant-infecting families ranked in the International Committee of the Taxonomy of Viruses (ICTV) (Adams et al., 2017). In 1906, disease caused by TSWV infection was observed for the first time. In 1919, it was named "spotted wilt of tomato" by Brittlebank (Stevens et al., 1995). It is ranked second among the top 10 plant viral diseases causing more than USD1 billion in yield losses annually in the most economically significant plants (German et al., 1995; Krishna Kumar et al., 1995; Mumford et al., 1996; Scholthof et al., 2011; Oliver and Whitfield, 2016). TSWV symptoms include necrosis, dieback, stunted growth, discoloration of young leaves, ring spots on the stem, yellowing, and size reduction of ripened fruit may also occur. However, particular symptoms differ from host to host. Small brown spots, dieback of young tips, and deteriorated red and orange patterns have been documented, reducing their economic significance and subsequent consumption of mature fruit. Among the currently described *Orthotospovirus* species, TSWV is the most investigated virus in Solanaceae crops (Adkins, 2000). Other orthotospoviruses with a more restricted geographic distribution and varying regional relevance include tomato chlorotic spot virus (TCSV), peanut bud necrosis virus (PBNV), iris yellow spot virus (IYSV), groundnut ringspot virus (GRSV), and impatiens necrotic spot virus (INSV). Some insect vectors, especially Thrips species (order: Thysanoptera, family: Thripidae) persistently and continuously spread orthotospoviruses from plant to plant, with efficient transmission by adults. Around 15 species of common Thrips have been reported as facultative vectors of TSWV (Rotenberg et al., 2015).

TSWV is also transmitted by seeds. Chemical management of TSWV is challenging due to the virus's transmissibility through Thrips species and its potential to change and adjust to various circumstances (Pappu et al., 2009). It can infect and damage tomato and pepper

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production worldwide to a great extent. While TSWV can also occur in Nordic countries, its impact on tomato growth may vary depending on various factors such as the specific virus strain, tomato variety, and environmental conditions.

TSWV is a tripartite in nature. Its genome is mainly divided into 3 sections, known as RNAs, as large (8.9 kilobase pairs (kb)), medium (4.8 kb), and small (2.9 kb). The L segment is monocistronic and encodes the viral complementary (VC) sense RNA-dependent RNA polymerase (*RdRp*). The M segment comprises progenitors of glycoproteins (Gn and Gc) and a nonstructural movement protein (*NSm*), which contributes to virus movement on the VC and viral (V) senses. The S segment contains the nucleocapsid *(N*) and the silencing suppressor protein (*NSs*) on VC and V RNAs (De Haan et al., 1991; Takeda et al., 2002; Bucher et al., 2003; Pappu et al., 2009). Plants identify invading organisms (viruses, bacteria, and fungi) via 2 types of immune receptors, i.e. attachment to the cell surface and intracellularly; these receptors activate the plant immune system. pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) is induced by activating a PAMP on the cell surface. The secondary immune response, effector-triggered immunity (ETI), is initiated by the development of disease-resistance genes (R genes) that target specific intracellular effectors in plants and is more efficient than PTI in limiting pathogenic transmission (Wang et al., 2020). Most of the R genes have an encoding nucleotide-binding site (NBS) domain and leucine-rich repeat (LRR) domain receptors, also known as NBS-LRR or NLR genes. Currently, most NLR genes that protect plants against viral invasion have been cloned in plants (Sun et al., 2020; Huang, 2021). Likewise, *Sw-5* and *Tsw* genes have been cloned and are commercially available for orthotospoviruses resistance breeding in Solanaceae plants. Moreover, the set of NLR genes providing TSWV resistance in tomatoes is still confined.

The development of resistant cultivars is among the greatest options for decreasing TSWV occurrence because of the favorable effects on the surroundings and human life. Many TSWV resistance loci have been identified, including *Sw1a*, *Sw1b*, *Sw2*, *Sw3*, *Sw4*, *Sw-5*, *Sw-6*, and *Sw-7* (Finlay, 1953; Spassova et al., 2001; Price et al., 2007; Saidi and Warade, 2008). It was reported that *S. peruvianum*'*s* resistance is controlled by the single dominant *Sw-5*, which is less isolate-specific, more stable, and useful for a wide range of breeding practices (Stevens et al., 1994; Pappu et al., 2009; De Oliveira et al., 2018). Recently, it was observed that the genes encoded for salicylic acid (SA) and Jasmonic acid (JA) expression levels, that SlTGA9 positively interacts and regulates Sl5R-1 to impact the SA and JA signaling pathways. These results demonstrated that the discovered Sl5R-1 gene controls TSWV resistance through its promoter, which interacts with transcription factor SlTGA9 (Qi et al., 2022). Likewise, *Tsw* gene resistance was found in wild relatives of *Capsicum chinense* (Boiteux, 1995). *Tsw* only confers resistance to TSWV, but *Sw-5* confers resistance to TCSV, CSNV, GRSV, INSV, and ANSV in addition to TSWV (Spassova et al., 2001; Price et al., 2007). *Sw-5* has been genetically mapped between the markers on chromosome 9, CT71, and CT220, while *Tsw* is mapped on chromosome 10 (Stevens et al., 1994). *Sw-5* locus has been linked to molecular markers [restriction fragment length polymorphism (RFLP), random-amplified polymorphic DNA (RAPD), cleaved amplified polymorphic sequences (CAPS), and sequence-characterized amplified region (SCAR)] (Chagué et al., 1996; Dianese et al., 2010; Ferrand et al., 2019).

TSWV resistance isolates are further classified into 2 types, i.e. resistance-inducing (RI) isolates, which can cause resistant cultivars to react hyper sensitively, and resistance-breaking (RB) isolates, which can overcome host resistance (Peiró et al., 2014; Turina et al., 2016). Following several years of continuous and widespread use of resistant tomato and pepper varieties, TSWV-RB isolates have emerged. The RB isolates appeared due to selective pressure caused by the use of resistant pepper and tomato cultivars (Thomas-Carroll and Jones, 2003; de Ronde et al., 2019). These isolates can overcome the resistance in tomato cultivars with the *Sw-5* gene, which was discovered for the first time in South Africa (Almási et al., 2017). The first incidences in Europe were reported in Spain and Italy in 2005. The TSWV-RB isolates that could silence the *Tsw* gene in resistant pepper cultivars were first reported from Brazil (Ferrand et al., 2019), followed by reports from Italy. Additionally, it has been observed in several other locations where tomato and pepper crops were frequently cultivated (Cho et al., 1995; Gordillo et al., 2008; Deligöz et al., 2014; Debreczeni et al., 2015; Almási et al., 2017; Batuman et al., 2017; Güneş and Gümüş, 2019; Jiang et al., 2022). A genetic component has been found to overcome *Sw-5* resistance in tomatoes encoded in the M segment. The *NSm* protein was defined to be the avirulent (AVR) determinant for the *Sw-5b* gene (Hoffmann et al., 2001; Hallwass et al., 2014). Moreover, TSWV's capacity to overcome *Sw-5* mediated resistance is linked to amino-acid changes at positions (C118Y) and (T120N) in the *NSm* protein encoded by the M segment of TSWV (Cho et al., 1995; Almási et al., 2017). The nucleotide sequences are vital for the clustering, phylogeny, diversity, and population studies of TSWV isolates, even though no known mutation in the *N* gene has been related to RB (Peiró et al., 2014). The genetic diversity and population structure of TSWV in Türkiye were discussed by Morca et al. (2022). Despite prior TSWV detections, the virus' population makeup is still unknown. Of 227 samples that

were collected in 2019–2020, 43.6% showed evidence of TSWV infection. The Turkish isolates were split into 2 separate clades by phylogenetic analysis, emphasizing genetic variation, and constrained gene flow. The study also showed that the N gene of TSWV is under substantial negative selection pressures. This study shed light on Türkiye's TSWV population dynamics.

The main objective of the current study was to evaluate TSWV infections, RB isolates, and conduct a phylogenetic analysis of TSWV isolates in correlation with tomato and pepper resistance genes under field conditions. For this purpose, suspicious tomato and pepper plants showing diverse symptoms of TSWV were collected from Türkiye. Plants were screened by serological and molecular methods for the incidence of virus and virus resistance genes. Moreover, the screening of the RB virus isolates was examined by nucleotide analysis. In addition, the obtained virus isolates were phylogenetically analyzed.

2. Materials and methods

2.1. Sample collection

The field survey was conducted, and 150 TSWV-suspected tomato and pepper samples were collected from different fields in Hatay, Mersin, and Niğde provinces based on symptoms from July–August of 2020–2021. The temperature was recorded as optimum at 28–40 °C. Samples were stored at –20 °C for further analysis.

2.2. Biological characterization

Double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) was used for the initial testing of the suspicious samples to detect the TSWV. In order to enhance the spread of the viral titer, tobacco plants were subsequently inoculated with positive samples that were chosen based on their host, symptoms, and geographic location. Following inoculation, plants that were evaluated as positive provided the virus for experimentation, allowing TSWV research to be conducted. This technique guaranteed that the detection and spread of the virus was done in a methodical and scientific manner. Leaf samples were manually ground using an inoculation buffer (pH 7.4) containing (0.199 g/L of KH_2PO_4, 1.14 g/L of Na_2HPO_4, and 0.1% of Na_2SO_3 and 1% PVP-40). Carborundum was applied in the mechanical inoculation of the seedlings at the 2–4 leaf stage. Mock inoculation using sterile water and carborundum were used to create the negative control plants. Each plant species was replicated 3 times. Inoculated plants were placed in a growth chamber with a 16-h photoperiod and a constant temperature of 25 °C for 4 weeks following inoculation, and symptom manifestation was monitored.

Up to 15–25 days post-inoculation (dpi), ELISA was performed on the inoculated and uninoculated top leaves. DAS-ELISA was performed 25–30 days after inoculations to check the TSWV infections based on Clark and Adams (1977) and Antisera's manufacturer's instructions for the monoclonal antisera of TSWV. After the ELISA, the plate was read by the ELISA reader, and numerical results of the ELISA were obtained. In addition to the serological detection, molecular tests were also performed to confirm the presence of TSWV in the tested plants.

2.3. Screening of resistance gene DNA extraction, polymerase chain reaction (PCR) analysis, and agarose gel electrophoresis

DNA was isolated from the leaves of TSWV-infected plants using the cetyltrimethylammonium bromide (CTAB) protocol with a few modifications (Doyle, 1991). First, 1 mL of 2% CTAB extraction buffer (0.2% of β-mercaptoethanol just before starting) was added to 0.5 g of fresh tomato and pepper leaves. The samples were vortexed and incubated at 60 °C for 55–60 min. Then, 24:1 chloroform:isoamyl alcohol was added to the solution invert and mixed for 10 s, and then centrifuged at 14,000 rpm for 3 min. The supernatant was transferred into a new tube, and 500 µL of cold isopropanol was added. The pellet was washed twice with 75% cold ethanol and then the pellet was eluted in Tris-ethylenediaminetetraacetic acid buffer. The eluted DNA was incubated at 37 °C for 1 h and stored at –20 °C.

DNA samples were subjected to PCR amplification using specific primers for the *Sw-5* and *Tsw* genes. For Sw-5, primers F (5'-AATTAGGTTCTTGAAGCCCATCT-3') and R (5'-TTCCGCATCAGCCAATAGTGT-3') generated amplicons of 574 bp (resistant) and 464 bp (susceptible), distinguishing between the 2 alleles. In the case of the *Tsw* gene, the SCAC568 CAPS marker was used with primers F (5'-GTGCCAGAGGAGGATTTAT-3') and R (5'-GCGAGGTGGACACTGATAC-3'), producing amplicons of 500 bp (resistant) and 568 bp (susceptible) to differentiate between the resistant and susceptible genotypes (Dianese et al., 2010). The PCR was done with 25 µL of a total volume containing 2 µL of DNA, 0.5 µL of 10 mM deoxyribonucleotide triphosphate (dNTP), 1 µL of 25 mM $MgCl_2$, 2.5 µL of 10X PCR buffer, and 0.5 µL of 10 µM of both primer pairs with 0.5 µL of 5 units µL of Taq DNA polymerase. PCR was carried out with denaturation at 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 53 °C for 1 min, and 72 °C for 1 min, and a final extension for 10 min at 72 °C. The PCR amplification was visualized after electrophoresis on 3% agarose gel and stained with Ethidium bromide under an ultraviolet (UV)-transilluminator. According to their locus, the genotypes were classified as homozygote and heterozygote resistant or susceptible.

2.4. Screening for RB TSWV isolate RNA extraction, reverse transcription-PCR (RT-PCR) assay, and sequencing

Total RNA was isolated using the rapid CTAB method with some modifications. Virus-specific primers were designed

and used for amplification and to obtain the partial *NSm*, *NSs*, and *N* segments separately. A 2-step RT-PCR protocol was used, and the first-strand cDNA was synthesized by RT of total RNAs using random hexamer primers and the ABM OneScript cDNA Synthesis Kit (Cat. No. G236; Applied Biological Materials Inc., Vancouver, Canada) instructions. The PCR was carried out in 25 µL of total reaction mix comprising 2 µL of cDNA, 0.5 µL of 10 mM dNTPs, 1 µL of 25 mM $MgCl_2$, 2.5 µL of PCR buffer, and 0.5 µL of 10mM of each primer set. PCR was performed by following steps; denaturation at 94 °C for 5 min, 40 cycles of 94 °C for 30 s, annealing for 1 min (specific primers), 72 °C for 1 min, and a final extension for 10 min at 72 °C (Table S1). The PCR products were visualized under UV light after electrophoresis on 1.5% agarose gel and stained with ethidium bromide under a UV-transilluminator. TSWV-specific gene regions were amplified from gene-specific primers and sequenced for checking TSWV-RB and TSWV-RI isolates. New sets of primers were developed herein due to challenges in amplifying the *NSm*, *NSs*, and *N* segments with the available primers. These segments were critical for this study, and the new primers were designed to enhance efficiency and reliability, addressing technical difficulties, and ensuring successful amplification (Table S1). Nucleotide comparisons of all of the isolates sequenced in this work were conducted using the Basic Local Alignment Search Tool (BLAST) and Molecular Evolutionary Genetics Analysis (MEGA X) (https://www.megasoftware.net/) package (Tamura et al., 2007; Kumar et al., 2018). Later, nucleotide sequences were aligned using Multiple Alignment using Fast Fourier Transform (MAFFT) software (https://mafft.cbrc.jp/alignment/software/) version 7 (Katoh and Standley, 2013).

2.5. Phylogenetic analysis

In total, 16 TSWV-positive samples from tomato and pepper plants showing resistance genes were selected and then amplified for the genomic regions of *NSs*, *NSm*, and *N*, and sequenced after purification using the QIAquick Purification Kit (Qiagen, Hilden, Germany) using an automated genetic analyzer (ABI3730, MedSanTek Company, Türkiye) using the same RT-PCR primer sets. Sequence comparisons of the nucleotides and amino acids were performed and used for the phylogenetic analysis to test the genetic variation among the TSWV isolates. Partial genomic alignments were generated for the tree construction, i.e. TSWV-*NSs*, TSWV-*N*, and TSWV-*NSm*, independently. Newick data of the aligned sequences were generated by IQ-TREE (http://iqtree.cibiv.univie.ac.at/) (Trifinopoulos et al., 2016). Independent methods were used to improve the robustness of the abovementioned results and tree topologies; phylogenetic trees were generated in parallel using the neighbor-joining (NJ) method, each with 1000 bootstrap replications. The phylogenetic NJ tree was constructed using Fig Tree version 1.4.4 (Tree Drawing Tool: http://tree.bio.ed.ac.uk/software/figtree/).

3. Results

3.1. Symptom observations

The study material collected comprised 40 pepper and 45 tomato plants from Hatay, 33 tomato and 7 pepper plants from Mersin, and 15 tomato and 10 pepper plants from Niğde. The most common virus-like symptoms were detected on plants exhibiting ring spots, necrosis on the stems, and yellowing on the leaves. Several plants showed various symptoms like brown circles, yellowing of the leaves, severe stem necrosis, and stunted growth. The symptoms are shown in Figure 1. Furthermore, to enhance the virus titer, infected tomato and pepper plants were selected based on DAS-ELISA analysis (data not shown) that showed virus-like symptoms from each region, which were inoculated in tobacco plants (*Nicotiana benthamiana* L.). Diverse symptoms started to appear after 2 to 3 weeks of inoculations as leaf deformation, leaf curling, and severe mosaics (data not shown). Furthermore, the DAS-ELISA method was employed to assess disease infection in leaf samples randomly collected from the inoculated tobacco cultivars. The ELISA revealed varying infection levels among the different cultivars. The highest infection rate was observed in Niğde pepper, with an 80% infection rate, closely followed by Hatay pepper at 78%. In contrast, Niğde tomato exhibited the lowest positive result, with an infection rate of only 38% (Table S2).

3.2. Screening of virus resistance gene

The codominant SCAR *Sw5-b* marker was used to confirm the plant's resistance or susceptibility status in tomatoes. Those with the homozygous genotype showed a band of 574 bp, those with the heterozygous genotype had bands of 574 bp and 500 bp, and those with the susceptible genotype showed a band of 464 bp, as expected. While in the case of the pepper CAPS, the $SCAC_{568}$ marker was used, and the samples showed 500 bp and 568 bp amplicons representing resistant and susceptible *Tsw* to select the resistant and susceptible genotypes. It was found that 110 samples were positive for DAS-ELISA and all of the positive samples were screened for virus resistance. As a result of the resistance analysis, 10 tomato and 6 pepper samples from Hatay, 12 tomato and 4 pepper samples from Mersin, and 8 tomato and 4 pepper samples from Niğde were found to carry resistance genes, even though they were showing intense TSWV symptoms (Figure 2).

3.3. Molecular characterization of the TSWV isolates

Several factors could break virus resistance such as genetic mutations and high temperatures. Therefore, after confirmation of the resistance gene incidence on the screened plants, the TSWV isolates were further

Figure 1. Samples showing diverse TSWV symptoms: (a) yellowing of the leaves; (b) leaf mosaic on the peppers; (c) yellowing of the veins; (d) ringspots on the peppers; (e) ringspots on the tomatoes, fruit deformation, and bronzing of the fruit; and (f) necrosis on the leaves.

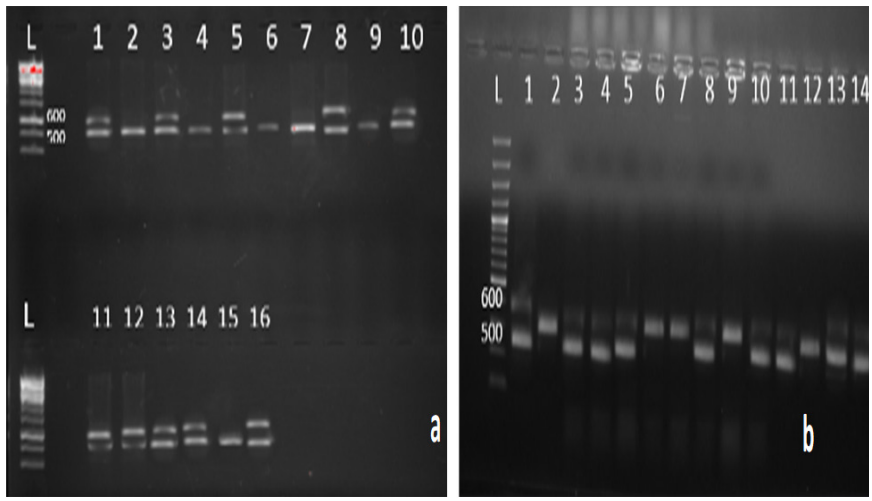

Figure 2. (a) Agarose gel results of the *Sw-5* primers. L: Thermo Scientific 100 bp plus DNA ladder. (b) Agarose gel electrophoresis results of the *Tsw* primers. L: Thermo Scientific 100 bp plus DNA ladder.

characterized for RB mutations. In total, 16 TSWV-positive samples were selected and amplified for partial genomic regions of TSWV using gene-specific primers (Table S3). The amplified samples were used for sequence analysis (Figure 3).

3.4. *NSs* and *NSm* amino acid comparison

BLAST analyses of the amino acids revealed that the *NSm* sequences of the putative RB and RI isolates had the highest nucleotide similarity with those in GenBank, with 90%–95% and 95%–97%, respectively. Alignment with the sequences of 16 Turkish TSWV isolates (4 samples from Hatay, 6 samples from Mersin, and 6 samples from Niğde) and previously described RB and RI isolates indicated variation in the amino acids of the *NSm* proteins of the selected Turkish isolates (Aramburu and Marti, 2003; Fidan and Sarı, 2019). In the GenBank RB isolates, 2

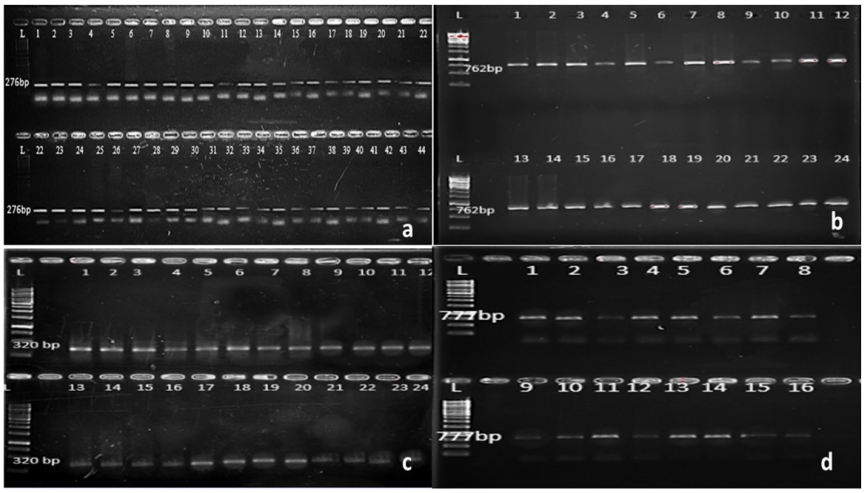

Figure 3. (a) Detection of TSWV by virus-specific primers, L: Thermo Scientific 1 kb plus DNA Ladder, samples 1 to 44, (b) Amplification of the samples by the TSWV-*NSm* primers, L: Thermo Scientific 1 kb plus DNA Ladder, samples 1 to 24, (c) Amplification of samples by TSWV-*NSs* primers, samples 1 to 24, (d) Amplification of the samples by the TSWV-N primers, samples 1 to 16.

substitutions (C118Y or T120N) in the *NSm* were found (Aramburu and Marti, 2003; Gordillo et al., 2008; Peiró et al., 2014; Debreczeni et al., 2015; Margaria and Rosa, 2015; de Ronde et al., 2019). TSWV-*NSs* protein is the avirulence factor of *Tsw*-mediated resistance. Furthermore, it was reported that the RB isolates appeared by various mutations (Margaria et al., 2004; Margaria et al. 2007; Margaria and Rosa 2015; Tentchev et al., 2011; Batuman et al., 2017; Kabaş et al., 2021). Therefore, to differentiate the *NSs* protein, the Turkish TSWV isolates were characterized and sequenced. The multiple sequence results showed that the identity ranged from 99% to 100%. No multiple mutations (D64N, I79T, C390Y, and S407T) were detected in the TSWV-*NSs* of the Turkish isolates. This observation was made through multiple sequence alignment analysis when comparing the *NSs* sequences of the Turkish isolates to a reference or other related sequences (such as those from RB and RI lines) (Figure 4).

3.5. Phylogenetic analysis

To establish the evolutionary framework, partial genomic sequences (*NSs*, *NSm*, and *N*) of the TSWV from samples collected in various regions, including Hatay, Mersin, and Niğde, were amplified. The selection of these regions was based on the phenotypic, biological, and molecular genotypic characteristics of the virus. Global TSWV retrieved from the National Center for Biotechnology Information (NCBI) GenBank were compared and subjected to phylogenetic analysis (Table). To construct a phylogenetic tree, *NSs* amplified sequences of the 16 TSWV isolates from Türkiye (4 samples from Hatay, 6 samples from Mersin, and 6 samples from Niğde) and 22 sequences from other countries (France, Italy, Iran, Hungary, Japan, China, Argentina, South Africa, and Spain) were used. The tree topology showed 3 main groups and several subgroups. Turkish isolates (Hatay and Mersin) were clustered together in the same clade and showed the highest similarity with French tomato TSWV isolate (RI: MN990971.1) and Italian pepper TSWV isolates (RB: DQ431237.1). The Hungarian isolate (KJ649610.1) was placed separately in 1 subgroup, as it did not show any close similarity with the other isolates referred to as the outgroup control. In the second leading group, all of the Turkish isolates were clustered together, showing more significant similarities, supported by higher bootstrap values. TCSV-*NSs* sequence (MH742961.1), referred to as the outgroup control, was distinctly separated from all of the other isolates, as shown in red in Figure 5A.

TSWV-N phylogenetic analysis was conducted, utilizing sequences obtained from 10 samples (4 from Hatay, 3 from Mersin, and 3 from Niğde) in conjunction with sequences obtained from GenBank. The analysis showed that these isolates exhibited a nucleotide identity of 85%–92% when compared to the other reference isolates used in constructing the phylogenetic tree. Based on the tree topology, it was concluded that the tree was divided into 3 major groups and several subgroups. All of the Turkish isolates were clustered together in 1 group that showed higher relevance among them. TSWV isolates from Korea and Australia (LC201874.1 and AY879111.1) were clustered in separate clades were not like any of the available isolates grouped as the outliers. TCSV-*NSs* sequence (MH742961.1), referred to as the outgroup

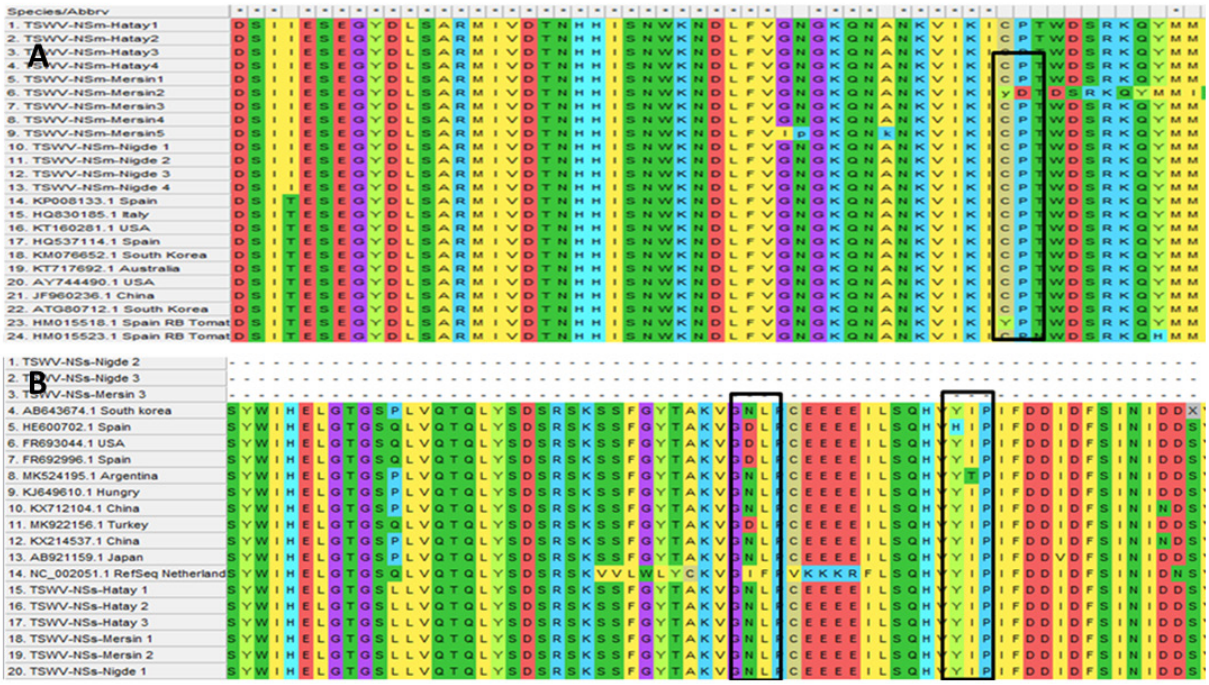

Figure 4. Sequence comparison of the TSWV *NSm* and *NSs* amino acid sequences from Turkish isolates and previously published RB and RI tomato and pepper isolates. The capacity to overcome *Sw-5* and *Tsw* resistance is connected with amino acid modifications to the (a) C118Y and T120N mutation shown in the black rectangular frame and (b) D64N and I79T shown in the black rectangular frame.

Table. Accession numbers retrieved from GeneBank utilized for multiple sequence alignments and phylogenetic analyses for the genome segments of *NSs, NSm*, and *N*.

Accession number	Isolates	Host	Country	Nature	Accession number	Isolates	Host	Country	Nature
TSWV-*NSs*									
MN990974.1	EM160322	Tomato	France	RI	MK922156.1	P11Ant	Pepper	Türkiye	RB
MN990973.1	EM160319	Pepper	France	RI	KX712104.1	YN65	Potato	China	RB
MN990971.1	EM160608	Tomato	France	RI	KX214537.1	YN53	Tobacco	China	RI
AB643674.1	TSWV-Pap	Pepper	South Korea	RI	AB921159.1	Mo	Thrips	Japan	RI
HE600702.1	P330	Pepper	Spain	RI	GU369752.1	T-1003	Tomato	Italy	RI
FR693044.1	TomUSA	Tomato	USA	RI	NC002051.1	CPNH9	TSWV	Netherlands	RefSeq
MK524195.1	TSWV-pA14	Pepper	Argentina	RB	DQ915947.1	Br20RB	Pepper	Brazil	RB
MG987618.1	TOS102	Tomato	Iran	RI	DQ915946	p105/2006RB	Pepper	Italy	RB
KJ649610.1	HUP3-2012	Pepper	Hungary	RB	MN990972	EM160251	Tomato	France	RI
DQ431237.1	p170	Pepper	Italy	RB	MN990970.1	EM160608	Tomato	France	RI
TSWV-*NSm*									

Table. (Continued)

HM015511.1	Cr1NL2	Tomato	Spain	RB	MN990969.1	EM160322	Tomato	France	RI
HM015523.1	Gr5TL1	Tomato	Spain	RB	MN990968.1	EM160319	Pepper	France	RI
HM015518.1	Llo2TL3	Tomato	Spain	RB	MN990967.1	EM160251	Tomato	France	RI
MH367503.1	TSWVAntRB	Tomato	Türkiye	RB	MN990966.1	EM160608	Tomato	France	RI
KP008133.1	PVR	Pepper	Spain	RB	MN990965.1	EM160039	Tomato	France	RI
KY495608.1	YN5576	Black nightshade	China	RI	MT241884.1	LN-HJL	Tropaeolum majus L	China	RI
FM163371	GA-1L	Tomato	Spain	RI	MG602672.1	Mum-A5	Chrysanthemum	Zimbabwe	RI
HQ537114	ALPA	Datura stramonium	Spain	RI	MN365035.1	YNHS	Peanut	China	RI
KM076652	LS3	Leonurus sibiricus	South Korea	RI	MK524206.1	TSWV-pA14	Pepper	Argentina	RB
AY744486	N-C3	Dahlia	USA	RI	MK524205.1	TSWV-pA5	Pepper	Argentina	RB
KT717692	TSWV-QLD1	Pepper	Australia	RI	MK524204.1	TSWV-pA24	Pepper	Argentina	RB
AY744490	NC-7	Tobacco	USA	RI	MK524203.1	TSWV-pA10	Pepper	Argentina	RB
JF960236	TSWV-YN	Tomato	China	RI	MK524202.1	TSWV-pA7	Pepper	Argentina	RB
ATG80712.1	Lk-1	Tomato and Amaranthus	Africa	RI	MK524201.1	TSWV-pA2	Pepper	Argentina	RB
NC_002050.1	ToSWVsMgp1	TSWV	USA	RefSeq	MK524200.1	TSWV-tA25	Pepper	Argentina	RB
MK524198.1	TSWV-tA15	Pepper	Argentina	RB	MK524199.1	TSWV-tA19	Pepper	Argentina	RB
TSWV-*N*									
KX710199.1	R367	Capsicum annuum	Bosnia	RI	DQ777619.1	NC-16	Tomato	USA	RI
MH172161.1	AART-TSWV	Tomato	Pakistan	RI	KY923205.2	TSWV-TP1	Tomato	Iran	RI
MK524185.1	TSWV-tA11	Tomato	Argentina	RI	DQ915948.1	Br20	Pepper	Brazil	RI
KT192625.1	TP-16	Datura stramonium	Türkiye	RI	DQ915947.1	Br20RB	Pepper	Brazil	RB
DQ523599.1	TSWV-L	Lettuce	USA	RI	DQ915946.1	P105\2006RB	Pepper	Italy	RB
MK605257.1	NS-TO22	Tomato	South Korea	RI	Z36882.1	T304	Pepper	Italy	RI
MK605256.1	NS-RP18	Pepper	South Korea	RI	KT160282.1	PA01	Pepper	USA	RI
KU884648.1	42-pep-zim	Pepper	Zimbabwe	RI	MT374760.1	Y8-1	Lettuce	China	RI
KP719131.1	TSWVA2	Pepper	Argentina	RB	MW067023.1	185-15	Tomato	Serbia	RI
AJ297608.1	DH37	Tomato	Bulgaria	RI	MW751977.1	B3	Pepper	Türkiye	RI
KY923205.2	TSWV-TP1	Tomato	Iran	RI	MW751976.1	B2	Pepper	Türkiye	RI
LC201874.1	Hamyang	Pepper	South Korea	RI					

*RI: Resistance inducing, RB: resistance breaking

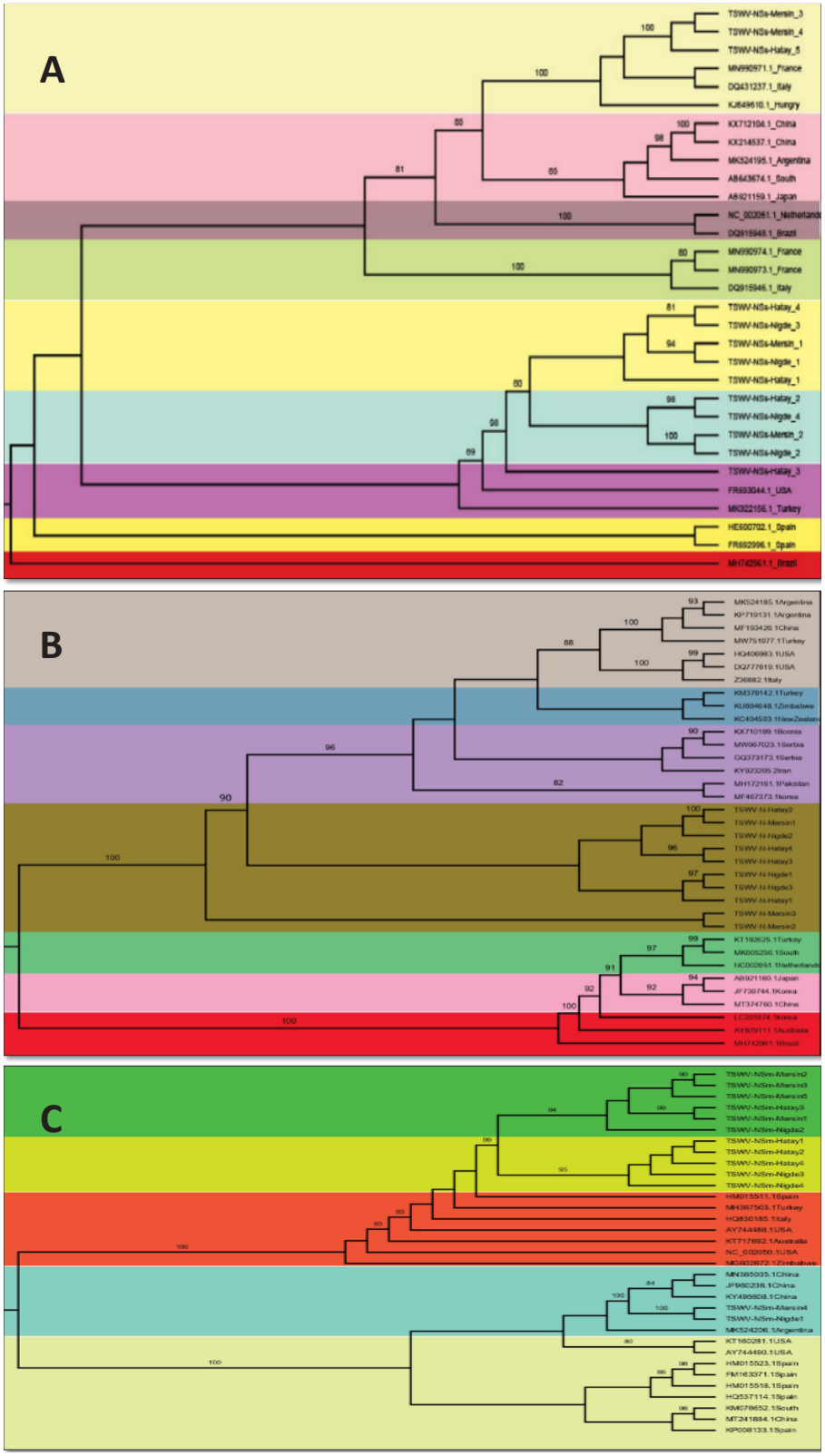

Figure 5. Phylogenetic analysis of the TSWV isolates by the NJ method using the (a) silencing suppressor protein (*NSs*), (b) nucleocapsid protein (N), and (c) movement protein (*NSm*). Bootstrap analysis was done with 1000 replications and values above 50 are given on the branch nodes.

control, was distinctly separated from all of the other isolates, as shown in red in Figure 5B. The *NSm* gene of the TSWV was identified as the Avr factor of the *Sw-5b* gene, with the Y118 or N120 residues critical for overcoming the hypersensitive response (HR) (Aramburu and Marti; 2003; Turina et al., 2016). Phylogenetic analysis of TSWV-*Nsm* was conducted using a combination of sequences, including those from 4 samples from Hatay, 5 samples from Mersin, and 4 samples from Niğde, along with sequences retrieved from GenBank. The *NSm* protein sequences exhibited a sequence similarity of 90%–95% with RefSeq, and these sequences were employed in the construction of the phylogenetic tree. The phylogenetic tree was divided into 4 main groups and different subgroups showing a bootstrap range >85. The Turkish isolates (Mersin and Niğde) shared a close resemblance with the TSWV-RB Argentine isolates (RB: MK524203.1) and TSWV-RI Chinese isolates (RI: KY495608.1). The Turkish isolates were clustered in separate groups and showed high similarities among them. In Figure 5C, samples in red indicate the outgroup showing no significant relationship or similarities with any of the TSWV isolates available. Moreover, it was previously reported that the C118Y point mutation impairs the production of the HR, resulting in the onset of systemic disease in infected plants (Fidan and Sarı, 2019).

4. Discussion

Among the management strategies for reducing TSWV damage, the establishment of resistant tomato and pepper cultivars is one of the most effective methods worldwide (Turina et al., 2016). The introduction of TSWV-RB isolates has been a serious concern and one of the most significant limiting factors for the effective production of pepper and tomato crops worldwide. Scientists are concentrating on rapid, sensitive, and dependable molecular approaches for detecting and eliminating serious viral diseases in order to enhance agricultural output quality (Sajid and Elçi, 2019). During 2020 and 2021, a comprehensive survey was carried out in various areas of Niğde, Mersin, and Hatay. This survey revealed notable decreases in crop yields due to the presence of highly impactful TSWV infections. Aside from the classic TSWV symptoms, extensive necrosis was observed on several plants, followed by stunting, foliar damage, yellowing, and leaf curls on the leaves and fruit. The serological analysis confirmed the incidence of TSWV in the collected samples. According to previous reports, TSWV infection has been detected in about 7.16% and 8.95% of pepper and tomato plants, respectively, in Türkiye's western Mediterranean regions (Yardımcı and Kılıç, 2009).

Due to the positive effects on the environment and human life, the development of resistant cultivars is one of the excellent options for reducing TSWV infections. The *Sw-5* and *Tsw* genes in tomato and pepper plants provide dominant resistance to TSWV (Stevens et al., 1995). In the current study, randomly selected TSWV-infected plants were further screened for resistance genes using SCAR and $SCAC_{568}$ markers. As a result, some plants (10 tomato and 6 pepper samples from Hatay, 12 tomato and 4 pepper samples from Mersin, and 8 tomato and 4 pepper samples from Niğde) were found to carry these R genes even though they exhibited TSWV symptoms. That could be attributed to the source collection and the variety of locations. The source collection was determined by randomly selecting samples from various tomato and pepper production locations that may contain resistance genes, resulting in plant diversity and the likelihood of these genes being present. Their geographical location may play a role in their resistance to viral infections. Even plants with known resistance genes may not always provide complete immunity against viral infections.

It underscores the necessity of comprehensive assessment, encompassing various variables, including the particular viral strain, host plant genotype, and prevailing environmental circumstances, in the examination of plant-virus interactions and the formulation of virus management approaches within agricultural practices (Roselló et al., 2001; Mandal et al., 2017; de Ronde et al., 2019). The widespread adoption of the *Sw-5* and *Tsw* alleles may have resulted in the positive selection, resulting in the emergence of RB isolates (Thomas-Carroll and Jones, 2003). The origination of RB isolates is independent of sequence variation, with the same single point mutation resulting in a resistance break. According to previous research, the C118Y mutation on the *NSm* proteins of orthotospovirus prevents *Sw-5* from initiating the HR, resulting in systemic viral infections in plants (Batuman et al., 2017; Roggero et al., 2002). TSWV-RB isolates have also been identified in Capsicum species containing the *Tsw* gene from Argentina and Türkiye (Gordillo et al., 2008; Peiró et al., 2014). TSWV-RB isolates breaking *Sw-5* resistance have been detected in Italy, Australia, California Spain, and Türkiye (Cho et al., 1995; Roselló et al., 2001; Thomas-Carroll and Jones, 2003; Deligöz et al., 2014).

It has been reported that there have been a few point mutations that occurred on TSWV-*NSs* (D64N, I79T, and C390Y) and TSWV-*NSm* on the locus (CT118Y). However, the amino acid alterations responsible for TSWV breakdown remain unknown (Hallwass et al., 2014). Researchers have demonstrated that position 79 was not the only amino acid position that characterized the RB (Margaria et al., 2007; Almási et al., 2017). Analysis of the *NSs* of the Turkish isolates did not show any alterations at I79T, D64N, and C390Y on the *NSs* protein. Furthermore, these isolates shared close relevance and similarities

with other RB isolates by sequence comparison and the phylogenetic tree. The results support the idea that the single and dominant viral resistance genes are very unstable and sensitive. They can also be affected by temperature, i.e. at or above 32 °C, the resistance is unable to function as previously reported. A novel class of temperature-sensitive RB TSWV isolates has been reported previously that elicit *Tsw*-mediated resistance at <28 °C but break it at ≥28 °C (Deligöz et al., 2014; Ferrand et al., 2019; Fidan and Sarı, 2019).

Identifying and characterizing any novel mutations, as well as investigating the genetic makeup of RB and RI isolates, can provide valuable insight into the mechanisms by which plant viruses overcome host resistance and evolve. Additional research and genetic analyses are required to clarify the relationship between TSWV genetic diversity and RB capacity, as well as to identify the possible importance of previously unrecognized mutations in the rise of RB and RI isolates (Scholthof et al., 2011; Hallwass et al., 2014; Fontana et al., 2020).

Several studies have shown that certain aggressive and virulent pathogens can overcome resistance conferred by resistance genes. This phenomenon is known as the breakdown of resistance or resistance erosion (Roselló et al., 2001; Fidan and Sarı, 2019). Moreover, researchers have identified new resistance genes, such as the NLR gene, Sl5R-1, which confers TSWV resistance through its promoter (Qi et al., 2022). In conclusion, it is vital to keep looking for new sources of resistance.

Multiple sequence alignment revealed that the obtained sequences of the TSWV-*NSs* genomic region of the Turkish isolates showed high similarity of 85%–89% resulting from nucleotide comparison with the Argentina RB isolates (MK524195.1), 89% with the Hungarian RB isolate (KJ8494610.1), 85% with the Antalya Türkiye RB isolate (MK922156.1), 85% with the Chinese RB isolate (KX712104.1), 87%–90% with the Brazilian and Italian RB isolates (DQ915947.1, DQ431237.1). The sequences obtained from the Turkish isolates from the TSWV-*NSm* sequences showed higher identity of 85%–95% with RB isolates from Spain, Antalya Türkiye, Italy, and Argentina (HM015511.1, HM015523.1, HM015518.1, MH367503.1, KP008133.1, and HQ830185.1, MK524206.1). The Turkish TSWV-N genomic sequences were compared with the retrieved sequences from NCBI and showed close identity of 85%–92% with RI Argentina, Tokat Türkiye, China (MK524185.1, KP719131.1, and MW751977.1). Moreover, these Turkish isolates showed less variability, at 33%, with the RB isolates from Brazil and Italy (DQ915947.1 and DQ915946.1), showing their distinct nature. Furthermore, the tree clusters contained RB and RI isolates, and no separation was identified, indicating that the genetic distances between isolates were unrelated to the phenotypes, showing the conserved nature of the *NSs* gene (Margaria and Rosa, 2015). The partial N gene sequences of the Turkish TSWV isolates revealed that specific isolates from various geographical parts of the country were more closely related to each other than the other GenBank isolates. To support previous studies, no substantial evidence was found for the involvement of the TSWV-N gene in the RB and RI isolates in the current study (Debreczeni et al., 2015).

Moreover, the TSWV-M section contained the *NSm* protein domain, which allowed the virus to migrate from cell to cell, showing variations among the Turkish isolates. The resistance conferred by the Sw-5 gene induced quick mortality of the contaminated tissues via HR responses and eliminated the chance of viral infection. The findings supported the notion that the development of an RB phenotype results from both factors, i.e. the effect of temperature and numerous characterized or uncharacterized single amino acid alterations distributed along the *NSs* and *NSm* sequence, which result in multiple sequence evolutionary events (Roselló et al., 2001; Almási et al., 2017; Ferrand et al., 2019). Furthermore, the *NSs* and *NSm* phylogenetic analyses revealed that the isolate clustering showed more linkage and similarities with the TSWV-RB and TSWV-RI phenotypes, consistent with earlier research that exhibited a pronounced degree of genetic similarity with TSWV isolates originating from various global regions (Soler et al., 2015; Petrović et al., 2021).

In the present study, the evaluation of TSWV infections, resistance genes, and phylogenetic analyses was performed. Three significant tomato and pepper growing locations in Türkiye (Niğde, Hatay, and Mersin) were screened for this purpose. Moreover, the outcomes of this investigation provided important fundamental information about the TSWV isolates' capacity to overcome resistance in pepper and tomato plants, which is a significant advancement toward identifying new and durable sources of TSWV resistance in these crops.

More investigations are needed to develop and execute effective management strategies for monitoring of the efficacy of resistance in different cultivars to combat TSWV and protect pepper and tomato production losses.

5. Conclusion

TSWV resistance cultivars are essential for TSWV management strategies in pepper and tomato. This resistance is primarily based on resistance genes that have deteriorated. It is known that high virus concentrations, high temperatures, and RB virus isolates can suppress these genes. According to the findings, proactive virus management strategies are critical in order to avoid infections. Implementing effective virus regulatory and mitigation measures before infections occur can significantly reduce crop damage and yield losses. It is

critical to conduct additional research to better understand the unidentified and unknown mutations that may be responsible for conquering resistance mechanisms and identifying novel resistance resources. Moreover, efficient control practices should be taken for Thrips management, which is a potential vector for TSWV. In recent years, innovative breeding tactics have outperformed traditional breeding approaches by providing a systematic overview of plant disease-resistance mechanisms against TSWV. The employment of innovative breeding tactics, such as RNA silencing mechanisms, targeted gene editing, and NLR artificial evolution, to create TSWV-resistant plants represents a new possibility in plant breeding, notably in disease resistance.

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Supplementary Material

Table S1. List of the specific primers designed for detection of the TSWV incidence and gene specific primers for *N*, *Nsm*, *and NSs* genes used in the RT-PCR.

Name	Sequence	Position	Reference
TSWV-New	F: 5'-ATTGCCTTGCAACCAATTC-3' R-5'- ATCAGTCGAAATG GTCGGCA-3'	------	Pérez et al., 2015
TSWV-NSs1	F:5' -ATGAACAGATCTGATATTAC-3' R: 5'- TCACTGCAAGAGAAT-3'	391-655 regions	This work
TSWV-*NSs*2	F: 5' -GGCAAAGTGAATGT-3' R: 5' -GCTGTCTCAACACTGT-3'	541-878 regions	This work
TSWV-*NSs*3	F: 5'-TTCTCAGCATGTGTATA-3' R: 5'-ACATTCACTTTGCC -3'	219-554 regions	This work
TSWV-*NSs*4	F: 5'-CACAATACTGTAATAAG-3' R: 5'-CAGGAGAAATTCAAAAG-3'	-------	Ferrand et al., 2019
TSWV-*NSs*5	F: 5'-CCGTCGACATGTCTTCAAGTGTT-3' R: 5'-GGCGGCCGCTTATTTTGATCCTGAA-3'	-------	De Ronde et al., 2019; Fidan and Sarı, 2019
TSWV-N1	F: 5'-ATGTCTAAGGTTAAGC-3' R: 5'-TTAAGCAAGTTCTGTGA-3'	1-777 region	This work
TSWV-N2	F: 5'-TACGGATCCGATGTCTAAGGTTAAGCTCAC-3' R: 5'-TTATCTCGAGTCAAGCAAGTTCTGCGAG-3'	-------	Ferrand et al., 2019
TSWV-*NSm*	F: 5'-ATGGATGCATCCAAAGGA-3' F: 5'-CTATATTTCATCAAAGGATAACTGAG-3'	-------	Ferrand et al., 2019

Table S2. ELISA results for the 150 samples of tomato and pepper investigated in this study.

Location	Species	Tested samples	TSWV-infected samples	TSWV-uninfected samples	Infection rate
Hatay	Tomato	45	30	15	66.67%
	Pepper	40	32	0,8	80.00%
Mersin	Tomato	33	30	03	90.90%
	Pepper	07	05	02	71.42%
Niğde	Tomato	15	05	10	33.33%
	Pepper	10	08	02	80%
Total	------	150	110	40	73.33%

Table S3. List of the samples along with their origins, hosts, and biological, serological, and molecular analyses results.

No.	Host	Origin	Symptoms	Serological test	Molecular test	No.	Host	Origin	Symptoms	Serological test	Molecular test
1	Tomato	Hatay	Necrosis	+	+	76	Pepper	Hatay	Necrosis	+	+
2	Tomato	Hatay	Leaf curl	+	+	77	Pepper	Hatay	Yellowing	+	+
3	Tomato	Hatay	Yellowing	+	+	78	Pepper	Hatay	Yellowing	+	+
4	Tomato	Hatay	Bronzing	+	+	79	Pepper	Hatay	Ringspot	+	+
5	Tomato	Hatay	Leaf mosaic	+	+	80	Pepper	Hatay	Chlorosis	+	+
6	Tomato	Hatay	Deformation	+	+	81	Pepper	Hatay	Yellowing	+	+
7	Tomato	Hatay	Necrosis	+	+	82	Pepper	Hatay	Ringspot	+	+
8	Tomato	Hatay	Necrosis	+	+	83	Pepper	Hatay	Chlorosis	+	+
9	Tomato	Hatay	Yellowing	+	+	84	Pepper	Hatay	Ringspot	+	+
10	Tomato	Hatay	Ringspot	–	–	85	Pepper	Hatay	Chlorosis	+	+
11	Tomato	Hatay	Chlorosis	+	+	86	Tomato	Mersin	Yellowing	–	–
12	Tomato	Hatay	Necrosis	–	–	87	Tomato	Mersin	Ringspot	+	+
13	Tomato	Hatay	------	+	+	88	Tomato	Mersin	Chlorosis	+	+
14	Tomato	Hatay	Yellowing	–	–	89	Tomato	Mersin	Necrosis	+	+
15	Tomato	Hatay	Bronzing	+	+	90	Tomato	Mersin	Chlorosis	+	+
16	Tomato	Hatay	Leaf mosaic	+	+	91	Tomato	Mersin	Yellowing	+	+
17	Tomato	Hatay	Deformation	+	+	92	Tomato	Mersin	Ringspot	–	–
18	Tomato	Hatay	Necrosis	–	–	93	Tomato	Mersin	Chlorosis	+	+
19	Tomato	Hatay	Spots	+	+	94	Tomato	Mersin	Mosaic	–	–
20	Tomato	Hatay	Yellowing	+	+	95	Tomato	Mersin	Deformation	+	+
21	Tomato	Hatay	Ringspot	+	+	96	Tomato	Mersin	Necrosis	–	–
22	Tomato	Hatay	Chlorosis	–	–	97	Tomato	Mersin	Ringspot	+	+

Table 3. (Continued)

23	Tomato	Hatay	Leaf mosaic	+	+	98	Tomato	Mersin	Yellowing	+	+
24	Tomato	Hatay	Deformation	+	+	99	Tomato	Mersin	Ringspot	+	+
25	Tomato	Hatay	Necrosis	+	+	100	Tomato	Mersin	Chlorosis	–	–
26	Tomato	Hatay	------	–	–	101	Tomato	Mersin	Yellowing	+	+
27	Tomato	Hatay	Yellowing	+	+	102	Tomato	Mersin	Ringspot	+	+
28	Tomato	Hatay	Ringspot	–	–	103	Tomato	Mersin	Chlorosis	+	+
29	Tomato	Hatay	Chlorosis	+	+	104	Tomato	Mersin	Yellowing	+	+
30	Tomato	Hatay	Leaf mosaic	–	–	105	Tomato	Mersin	Ringspot	–	–
31	Tomato	Hatay	Deformation	+	+	106	Tomato	Mersin	Chlorosis	+	+
32	Tomato	Hatay	Necrosis	+	+	107	Tomato	Mersin	Yellowing	+	+
33	Tomato	Hatay	------	+	+	108	Tomato	Mersin	Yellowing	+	+
34	Tomato	Hatay	Yellowing	–	–	109	Tomato	Mersin	Ringspot	–	–
35	Tomato	Hatay	Ringspot	+	+	110	Tomato	Mersin	Chlorosis	+	–
36	Tomato	Hatay	Chlorosis	–	–	111	Tomato	Mersin	Yellowing	+	+
37	Tomato	Hatay	Yellowing	+	+	112	Tomato	Mersin	Ringspot	+	+
38	Tomato	Hatay	Ringspot	–	–	113	Tomato	Mersin	Chlorosis	–	–
39	Tomato	Hatay	Chlorosis	+	+	114	Tomato	Mersin	Yellowing	+	+
40	Tomato	Hatay	Yellowing	+	+	115	Tomato	Mersin	------	+	+
41	Tomato	Hatay	Ringspot	+	+	116	Tomato	Mersin	Yellowing	+	+
42	Tomato	Hatay	Chlorosis	–	–	117	Tomato	Mersin	Ringspot	+	+
43	Tomato	Hatay	Yellowing	+	+	118	Tomato	Mersin	Chlorosis	+	+
44	Tomato	Hatay	Ringspot	–	–	119	Pepper	Mersin	Yellowing	–	–
45	Tomato	Hatay	Yellowing	+	+	120	Pepper	Mersin	------	+	+
46	Pepper	Hatay	Deformation	+	+	121	Pepper	Mersin	Necrosis	+	+

Table 3. (Continued)

47	Pepper	Hatay	Yellowing	+	+	122	Pepper	Mersin	------	+	+
48	Pepper	Hatay	Bronzing	+	+	123	Pepper	Mersin	Yellowing	–	–
49	Pepper	Hatay	Leaf mosaic	–	–	124	Pepper	Mersin	Ringspot	+	+
50	Pepper	Hatay	Deformation	+	+	125	Pepper	Mersin	Chlorosis	+	+
51	Pepper	Hatay	Necrosis	–	–	126	Tomato	Niğde	Mosaic	–	–
52	Pepper	Hatay	Diebacks	+	+	127	Tomato	Niğde	damage	–	–
53	Pepper	Hatay	Yellowing	–	–	128	Tomato	Niğde	Necrosis	+	+
54	Pepper	Hatay	Ringspot	+	+	129	Tomato	Niğde	------	–	–
55	Pepper	Hatay	Chlorosis	+	+	130	Tomato	Niğde	Yellowing	+	+
56	Pepper	Hatay	Necrosis	+	+	131	Tomato	Niğde	Ringspot	+	+
57	Pepper	Hatay	------	–	–	132	Tomato	Niğde	Chlorosis	+	+
58	Pepper	Hatay	Yellowing	+	+	133	Tomato	Niğde	Yellowing	+	+
59	Pepper	Hatay	Bronzing	–	–	134	Tomato	Niğde	Ringspot	+	+
60	Pepper	Hatay	Leaf mosaic	+	+	135	Tomato	Niğde	Chlorosis	+	+
61	Pepper	Hatay	Deformation	+	+	136	Tomato	Niğde	Yellowing	–	–
62	Pepper	Hatay	Necrosis	+	+	137	Tomato	Niğde	Ringspot	+	+
63	Pepper	Hatay	------	+	+	138	Tomato	Niğde	Chlorosis	+	+
64	Pepper	Hatay	Yellowing	+	+	139	Tomato	Niğde	Yellowing	–	–
65	Pepper	Hatay	Ringspot	+	+	140	Tomato	Niğde	Yellowing	+	+
66	Pepper	Hatay	Chlorosis	+	+	141	Pepper	Niğde	Ringspot	+	+
67	Pepper	Hatay	Leaf mosaic	+	+	142	Pepper	Niğde	Chlorosis	+	+
68	Pepper	Hatay	Deformation	+	+	143	Pepper	Niğde	Yellowing	–	–
69	Pepper	Hatay	Necrosis	+	+	144	Pepper	Niğde	Ringspot	–	–
70	Pepper	Hatay	------	+	+	145	Pepper	Niğde	Chlorosis	+	+

Table 3. (Continued)

71	Pepper	Hatay	Yellowing	+	+	146	Pepper	Niğde	Yellowing	+	+
72	Pepper	Hatay	Ringspot	+	+	147	Pepper	Niğde	------	+	+
73	Pepper	Hatay	Chlorosis	+	+	148	Pepper	Niğde	Chlorosis	+	+
74	Pepper	Hatay	Leaf mosaic	+	+	149	Pepper	Niğde	Yellowing	+	+
75	Pepper	Hatay	Deformation	+	+	150	Pepper	Niğde	Ringspot	+	+

*+: present, -: Absent