

Exploring the weed host range of resistance-breaking variants of tomato spotted wilt orthotospovirus (TSWV) across life cycles in Türkiye

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Abstract: Tomato spotted wilt orthotospovirus (TSWV) is destroying tomato and pepper resistance all over the world, including Antalya (Türkiye). Two greenhouses that show infection of TSWV in the Serik (coastal) and Elmalı (highland) areas were chosen for research between 2019 and 2021 to better understand the disease's life cycle. During the surveys, we focused on weed hosts to better understand TSWV disease's cycle. TSWV infection was determined in 58 peppers, 34 tomatoes, 270 weeds, and 20 other vegetable samples. Weed samples revealed essentially no symptoms, however, grown plants showed classic TSWV symptoms. The *Asteraceae* family had the highest infection rate among infected weeds, followed by weed species from the *Poaceae* and *Solanaceae* families. In addition, to determine the viral strain in the infected plant samples, qRT-PCR and Melt-curve analysis were done using a specially designed primer pair for the study. This primer identifies the point mutation on the NSm-movement protein in the viral genome's medium segment. The non-resistance breaking isolate of TSWV was included in the optimisation studies to evaluate differences between the two isolates at two thermal melting values established by this comparison. These findings demonstrated that the kits, procedures, and primers employed in this investigation can serve as a quick and reliable diagnostic tool for identifying TSWV isolates and that weeds are a key intermediate source for new TSWV infection, as confirmed by sequence data.

Keywords: resistance breaking isolate; TSWV; diagnostic kit; qRT-PCR; weed

Tomato spotted wilt orthotospovirus (TSWV) is a widespread problem in tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum* L.), and it is a significant pathogen that causes economic loss in these crops. This RNA virus is a member of the *Orthotospovirus* genus, which is part of the *Tospoviridae* family and the order *Bunyavirales* (Herath et al. 2020). The pathogen has been found in over 1 500 plant species, ranging from weeds to cultivated plants, across various tropical and subtropical parts of the world (Oliver & Whitfield 2016).

TSWV is a consistently propagative pathogen in nature, spreading quickly and easily through

at least 15 different thrips species, including the common *Frankliniella occidentalis* (Pergande) (*Thysanoptera: Thripidae*). The acquisition and transmission of thrips is connected with the first and second instar larval stages, and the rate decreases with their development (Riley et al. 2011). TSWV and similar plant viruses are spreading due to increased international trade between countries involving trading seeds and fresh vegetables (Pappu et al. 2009). This rationale, combined with recent climate change, has resulted in the emergence of novel *Orthotospovirus* in countries such as Türkiye and a rapid increase in the incidence

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and severity of *Orthotospovirus*-caused diseases. Furthermore, TSWV can survive in nature on biennial and perennial plant species during the winter and cause disease the next season (Takacs et al. 2006). Numerous studies show that emerged strains of TSWV can overcome host-plant resistance, emphasising the importance of controlling weeds, which are a source of TSWV inoculum, and highlighting the need for effective insecticides that can be used on thrips vectors (Batuman et al. 2017; Ferrand et al. 2019; Kabas et al. 2021).

The study's main subject was to discover which virus hosts completed their life cycle and identify the dominant isolate in survey areas, particularly when tomatoes and peppers (the main hosts) are not produced. As a result, weed hosts of TSWV were found during the investigation, and attempts were undertaken to build an effective and long-term complete understanding of TSWV outbreaks.

MATERIAL AND METHODS

Plant survey and collection samples

Two grebenhouses (one for pepper, the other for tomato) with TSWV infection were selected from two districts within Antalya province. The survey was planned separately for both the districts according to the climatic conditions and the breeding season. The first zone, Serik (coastland), had nine months of agricultural activity coupled with the solarisation treatment. The second zone, Elmali (highland), also had nine months of agricultural activity with no weed solarisation treatment. To better understand the characteristics of winter weeds that serve as non-crop hosts for TSWV and insect vectors, greenhouses of tomato and pepper in two areas were monitored at regular intervals between 2019–2021. Species identification of

weeds was made by Dr. Yasin Emre Kitis at Akdeniz University Weed Science Laboratory by using the identification keys of the Flora of Türkiye (Davis 1965–1985; Davis et al. 1988; Güner et al. 2000). TSWV-infected plant material, healthy-appearing cultivars, and weeds with and without symptoms were collected. Photographs were taken of each sample, which were then coded. During the surveys, 2–3 leaf and fruit samples were collected from tomato and pepper plants exhibiting TSWV-like symptoms (Figures 1 and 2).

The samples were transported to the laboratory in an icebox. Fruit and leaves of tomato and pepper plants were used as inoculum for further experiments. Some of the collected samples were kept at 4 °C for short periods before being analysed, while others were kept at –80 °C for longer periods for possibly subsequent testing.

The investigations were conducted during the summer and winter seasons of the year (during the production season and after harvesting of the primary crops in the absence of the main host). Table 1 displays all of the obtained samples. These samples were subjected to serological and molecular tests in the Virology Laboratory of Akdeniz University's Plant Protection Department.

DAS-ELISA analysis

The Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA) was performed in accordance with Clark and Adams' (1977) method and the protocol provided by the antisera manufacturing business. Bioreba Company Reinach, Switzerland, provided TSWV antisera. At 30 and 50 minutes following substrate addition, absorbance values at 405 nm (A405) were determined using an ELISA reader. All positive samples had a value at least three times higher than the negative control values.

Table 1. Sample details collected from tomato and pepper growing areas of Antalya

Plant type	District/ village	Greenhouse (ha)	No. of samples	District/ village	Greenhouse (ha)	No. of samples	Grand total
Pepper			25			33	58
Tomato			13			21	34
Other (*)	Serik / Cakallik	0.25	11	Elmali / Golova	0.20	9	20
Weed			185			85	270
Total			234			148	382

*Other: some vegetables (tomatoes, peppers, eggplant, chard, corn, beans, parsley, and okra) grown for fresh consumption by greenhouse owners

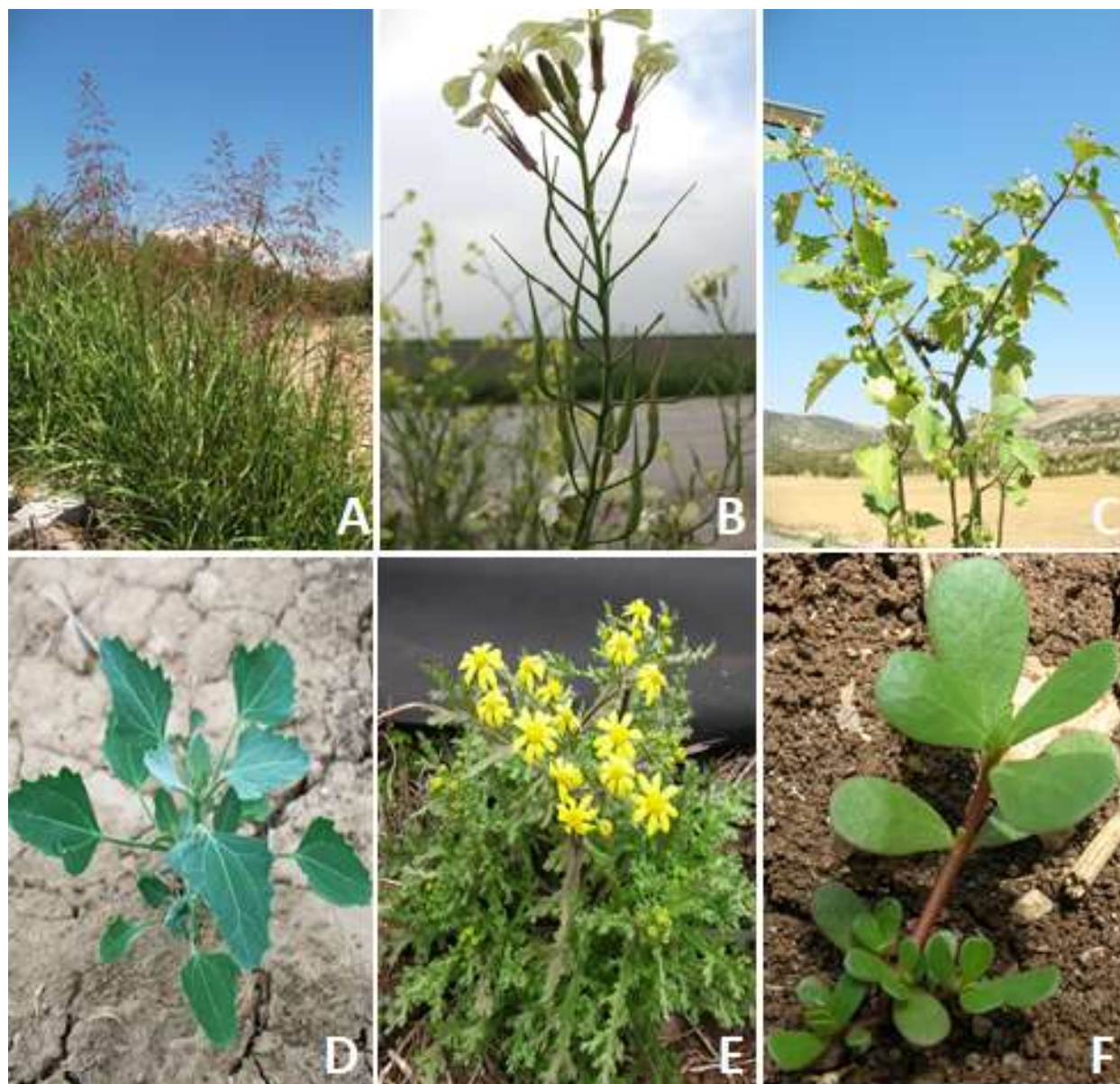


Figure 1. Weeds are suspected TSWV hosts grown in and around the greenhouses

(A) *Sorghum halepense*, (B) *Raphanus raphanistrum*, (C) *Solanum nigrum*, (D) *Chenopodium album*, (E) *Senecio vulgaris*, (F) *Portulaca oleracea*

Primer development

The change of the C amino acid at position 118 (C118Y) to Y amino acids and the T amino acid at position 120 (T120N) to N amino acids was determined by comparing the sequences of different Resistant Breaking (RB) TSWV isolates (Padmanabhan et al. 2019). This knowledge was useful in the development of primers. Thus, 100 forward and reverse base pairs were chosen from NCBI to target the 118th amino acid mutation area in the medium segment of ten resistance-breaking isolates and ten non-resistance-breaking isolates. Primer development investigations were carried

out to discover the gene region where point mutations were detected, analyse Sanger Sequencing data, and understand which TSWV isolate was detected by frequent testing with these primers and employ them in our laboratory. The primers were created using the Primer3 web (version 4.1.0). The Mega 11: Molecular Evolutionary Genetics Analysis (version 11) (Tamura et al. 2021) was used to analyse the sequence data.

Molecular determination of TSWV in samples

Total nucleic acid (RNA) was isolated from different plant parts (leaf, fruit, stem, and flower) us-

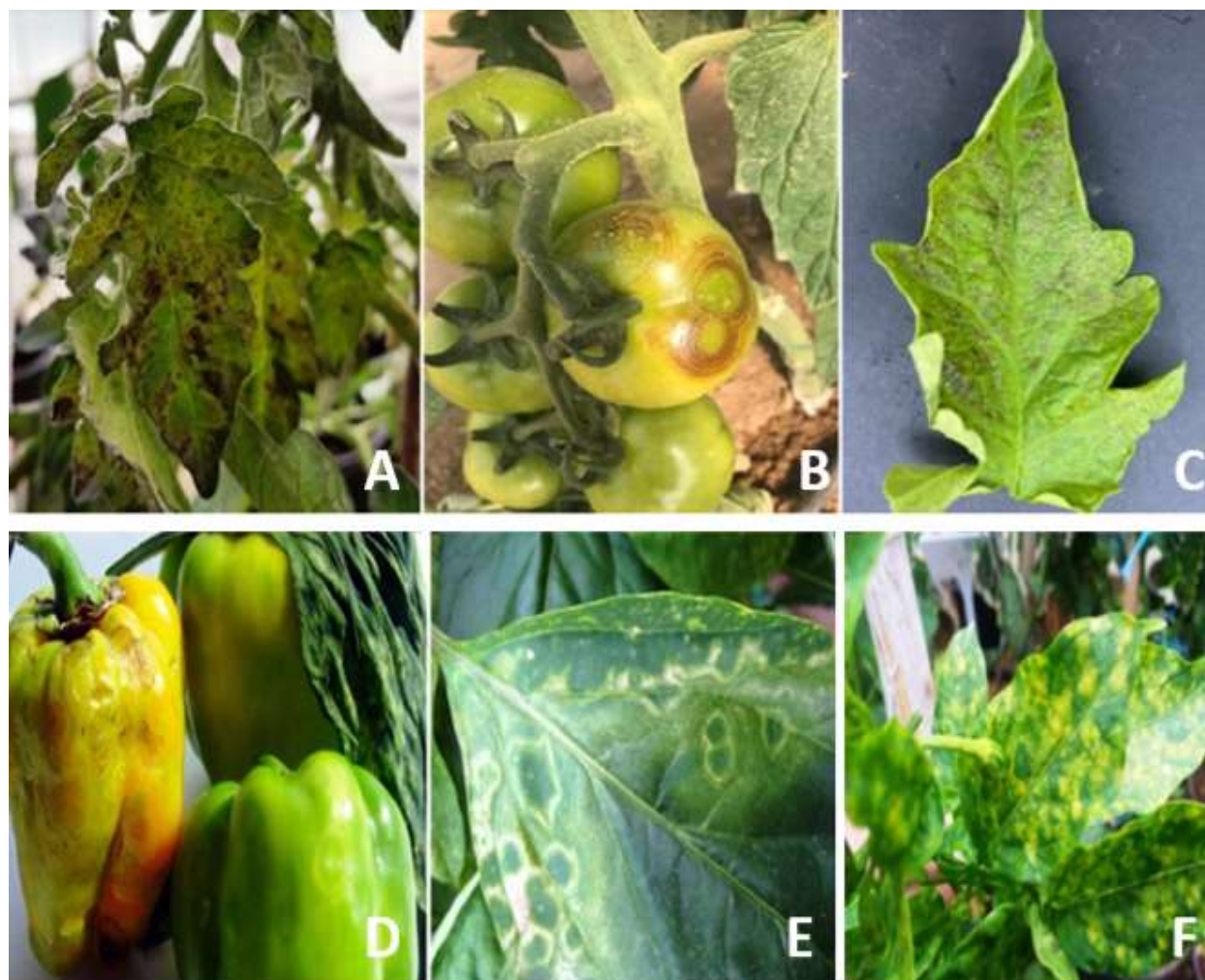


Figure 2. Characteristic symptoms of TSWV on tomato (A–C) and pepper (D–F)

ing a method developed by Presting et al. (1995). The obtained RNA was measured using a spectrophotometer (A260/280 1.8–2.0), and their concentration was adjusted to 200 ng/μL. After RNA optimisation, the RT-PCR method was carried out using Thermo Fisher Scientific Verso 1-Step RT-PCR Kit ReddyMix. In this study, primers L2TSWVF (5'-AATTGCCTTGCAACCAATTC-3') and L1TSWVR (5'-ATCAGTCGAAATGGTCG-GCA-3') were utilised for Tomato Spotted Wilt Orthospovirus (TSWV) detection, following the methodology established by Mumford et al. 1994, while newly designed primers TSWVF2 (5'-GC-CGAAAGGATGAAGGTCC-3') and TSWVR1 (5'-TGTCCTTGACCCTTCAGGA-3') were employed specifically for mutation detection in the same study. The electrophoresis gels were scanned with BiodocAnalyze (version 2.2) computer program attached in gel documentation system (Bio-

metra gel documentation, Germany). RT-PCR results from 13 distinct weed and tomato plant isolates that do not break resistance (NRB) to TSWV were prepared using primers specific to the medium segment of TSWV and sent to a commercial company (Nanogen Medical, Türkiye) for custom sequencing, followed by sequence analysis.

Quantitative RT-PCR (qRT-PCR) studies

A high-resolution melting (HRM) study was done on the old isolate of NRB-TSWV and the TSWV isolates, which were recovered from 13 weeds and diverse crop plants. Before the Real-time investigation, RNAs were extracted from infected plants using the NEB Luna Universal Probe One-Step RTqPCR Kit (Catalog No. E3006, NEB, Ipswich, MA, US). According to the user's instructions, the Melt-Curve step was executed at the end of the cycles. The reverse transcription process included a pre-

denaturation step at 55° C for 10 min, followed by cDNA synthesis at 95 °C for 1 min, denaturation at 95 °C for 10 s, and 30 s of elongation at 40–45 cycles, with a final melt curve stage ranging 60–95 °C for 15–60 s. The concentration of the samples in qRT-PCR was determined and diluted to the same value as directed. Comparisons were made using the Sanger sequencing results with the appropriate controls to validate the HRM result of RB-TSWV and NRB-TSWV isolates with different peaks in the Melt-Curve stage. The identification and dis-

crimination of alleles were carried out based on the temperature-changing difference curves produced by critically monitoring the Real-Time PCR data. The likelihood of mutation occurring in the medium segment of the isolates was explored in this investigation. At an annealing temperature of 60 °C, a final volume of 20 L was measured using a standard technique provided by the manufacturer. After that, amplification curves were obtained. For cluster detection, the melting temperature difference threshold (T_m), which determines the lowest T_m

Table 2. Potential host species that are showing symptoms that can be associated with TSWV infection

Sample No.	Plant species	No. of samples	Symptom	Sample No.	Plant species	No. of samples	Symptom
Amaranthaceae				Fabaceae			
Co1	<i>Amaranthus retroflexus</i> L.	3	–	24	<i>Medicago minima</i> (L.) Bartal	3	–
2	<i>Amaranthus chlorostachys</i> Willd.	13	+	25	<i>Medicago</i> sp.	5	–
3	<i>Chenopodium album</i> L.	17	–	Lamiaceae			
4	<i>Spinacia oleracea</i> L.	3	–	26	<i>Ocimum basilicum</i> L.	3	–
Asteraceae				27	<i>Thymus vulgaris</i> L.	1	–
5	<i>Calendula officinalis</i> L.	1	–	Malvaceae			
6	<i>Cirsium arvense</i> (L.) Scop.	2	+	28	<i>Malva parviflora</i> L.	17	–
7	<i>Conyza canadensis</i> (L.) Cronquist	8	+	Poaceae			
8	<i>Lactuca serriola</i> L.	16	+	29	<i>Digitaria sanguinalis</i> L.	5	–
9	<i>Matricaria chamomilla</i> L.	4	–	30	<i>Sorghum halepense</i> (L.) Pers.	9	–
10	<i>Senecio vulgaris</i> L.	2	+	Polygonaceae			
11	<i>Silybum marianum</i> (L.) Gaertn.	1	–	31	<i>Polygonum aviculare</i> L.	10	–
12	<i>Sonchus asper</i> (L.) Hill.	22	+	32	<i>Rumex</i> sp.	1	–
13	<i>Sonchus oleraceus</i> L.	13	–	Portulacaceae			
14	<i>Tagetes patula</i> L.	2	–	33	<i>Portulaca oleracea</i> L.	20	–
15	<i>Xanthium strumarium</i> L.	2	–	Rubiaceae			
Brassicaceae				34	<i>Galium aparine</i> L.	1	–
16	<i>Capsella bursa-pastoris</i> (L.) Medik.	10	–	Scrophulariaceae			
17	<i>Raphanus raphanistrum</i> L.	1	+	35	<i>Veronica hederifolia</i> L.	2	+
Caryophyllaceae				Solanaceae			
18	<i>Cerastium dichotomum</i> L.	3	–	36	<i>Datura stramonium</i> L.	13	–
19	<i>Stellaria media</i> (L.) Vill.	8	–	37	<i>Solanum nigrum</i> L.	15	+
Convolvulaceae				Urticaceae			
20	<i>Convolvulus arvensis</i> L.	9	–	38	<i>Urtica dioica</i> L.	2	–
Cyperaceae				Zygophyllaceae			
21	<i>Cyperus rotundus</i> L.	14	–	39	<i>Tribulus terrestris</i> L.	3	–
22	<i>Cyperus</i> sp.	2	–				
Euphorbiaceae							
23	<i>Euphorbia helioscopia</i> L.	4	–				

differences across samples for which the software would call different clusters, was set to 0.25 °C.

RESULTS

TSWV was found on a variety of weeds during surveys done over the course of two years (2019–2021). Serological and molecular studies were performed on 382 plant samples, including 270 weeds and 112 vegetables (tomatoes, peppers, eggplant, chard, maize, beans, parsley, and okra). Thirty-nine distinct weed species from 18 plant families were discovered in the investigated locations, and some common weed species were identified as potential TSWV hosts (Table 2).

TSWV detection using serological methods

The DAS-ELISA results revealed that 19 of 39 weed species were infected with TSWV (Table 3).

Table 3. Results of ELISA were carried out with collected weeds in two different locations for TSWV detection

Weed species	Coastland (Serik district)	Highland (Elmali district)
<i>Amaranthus chlorostachys</i> Willd.	+	+
<i>Cirsium arvense</i> (L.) Scop.	–	+
<i>Convolvulus arvensis</i> L.	+	+
<i>Conyza canadensis</i> (L.) Cronquist.	+	–
<i>Digitaria sanguinalis</i> (L.) Scop.	+	+
<i>Lactuca serriola</i> L.	–	+
<i>Medicago</i> sp.	+	–
<i>Polygonum aviculare</i> L.	+	+
<i>Portulaca oleracea</i> L.	+	–
<i>Raphanus raphanistrum</i> L.	–	+
<i>Senecio vulgaris</i> L.	–	+
<i>Solanum nigrum</i> L.	+	+
<i>Sonchus asper</i> (L.) HILL.	+	+
<i>Sonchus oleraceus</i> L.	+	–
<i>Sorghum halepense</i> (L.) Pers.	+	–
<i>Tagates patula</i> L.	–	+
<i>Tribulus terrestris</i> L.	+	–
<i>Veronica hederifolia</i> L.	–	+
<i>Xanthium strumarium</i> L.	+	+

The positive value recorded at 405 nm was 2.862, and samples with absorbance values three times higher than the negative value of 0.210 were classified as positive

Although 9 of the 39 plant species were positive for ELISA, they do not show typical TSWV symptoms (Figure 3). These signs included mild yellowing of the leaves, a mosaic pattern and deformation, stunting, bruising on the petioles, chlorotic and necrotic lesions on the leaves, and mottling. On the other hand, half of the weed samples or species examined for TSWV showed no evident signs of infection, and 20 weed species were confirmed to be disease-free.

The discovery of other species that were positive for infection despite being asymptomatic led to their classification as occult hosts that should be regarded more carefully. *Sonchus oleraceus*, *Xanthium strumarium*, *Tagates patula*, *Medicago* sp., *Sorghum halepense*, *Digitaria sanguinalis*, *Portulaca oleracea*, *Convolvulus arvensis*, *Tribulus terrestris*, and *Polygonum aviculare* are the species involved.

TSWV detection using nucleic acid-based molecular methods

HRM (High-resolution melting) analysis. Within the scope of the investigation, a unique primer pair was created to discriminate between the NRB-TSWV and RB-TSWV genotypes of TSWV in the region of mutation incidence for qRT-PCR tests. The isolate that did not show TSWV-NRB peaked at 82.0 °C T_m after HRM analysis, while the isolate that did show TSWV-RB peaked at 81.4 °C (Figure 4). When the sequence data from these two isolates were examined, it was discovered that the kits and primers utilised could form a dependable diagnostic test for detecting the mutation.

Some of the analysed samples were infected with TSWV, indicating that weeds can be an important alternate host and intermediate source for TSWV throughout the year (four seasons). It is worth mentioning that the major hosts (pepper and tomato) were not present in the greenhouse from July to October, although weed samples were positive for TSWV. In such instances, polyphagous-fed thrips prefer to live on weeds and spread the virus while feeding. Based on this observation, surveys were done often during the summer and early autumn. The findings revealed that isolates with point mutations in their viral movement proteins can be transmitted at any time of year. It has found that more abundant load in samples that were collected in the summer.

Following the PCR experiments, the MEGA11 program was used to study the phylogenetic analysis of weed TSWV isolates whose sequence analyses were obtained by sequencing service procurement. Se-

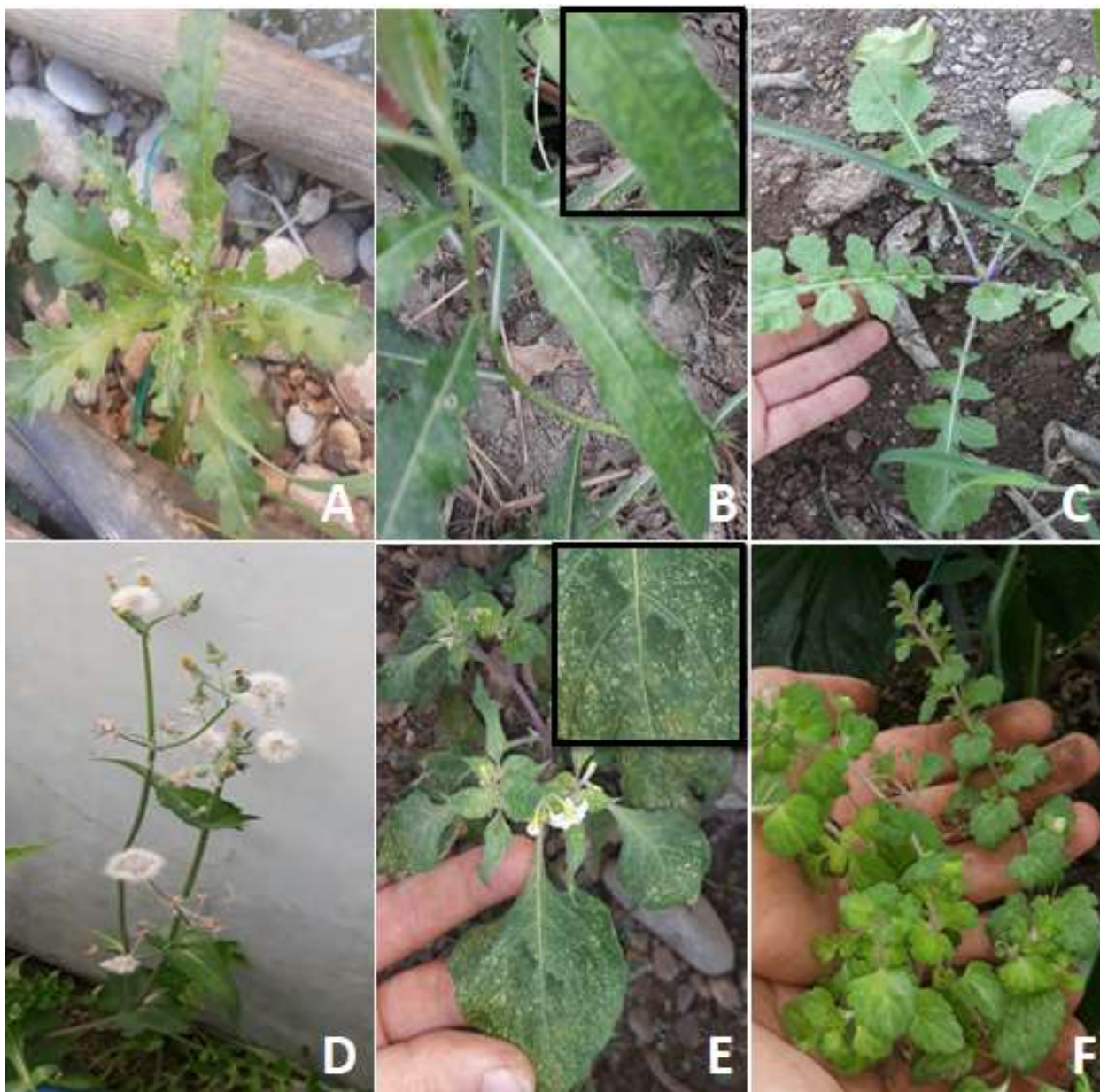


Figure 3. Viral symptoms observed on weeds

(A–B) light yellowing and mosaic symptoms on *Senecio vulgaris* and *Convolvulus arvensis* leaves, respectively, (C) dark green spots on *Raphanus raphanistrum*, (D) leaf margin yellowing on *Sonchus asper*, (E) leaves brittleness and mottling on *Solanum nigrum*, (F) mosaic appearance and yellowing on *Veronica hederifolia*

quence analysis results revealed a similar situation in samples 13 (old isolate that did not break resistance; contains G at nucleotide 354), 7, and only in one of the weeds, *Solanum nigrum* (Black nightshade, sample 7). Based on these data, 98% of the infected weed isolates were classified as resistance-breaking isolates. The findings revealed that 20 of the 34 TSWV-positive samples came from the coastland district and 14 from the highland district. In both regions, seven species were identified as common hosts (Table 3).

Eleven of these species were determined to be annual, five to be biennial, and three to be perennial. This distribution varies with the sampling site, and no uniform distribution was identified.

DISCUSSION

Tomato spotted wilt orthotospovirus (TSWV) is a widespread problem in tomato and pepper as well

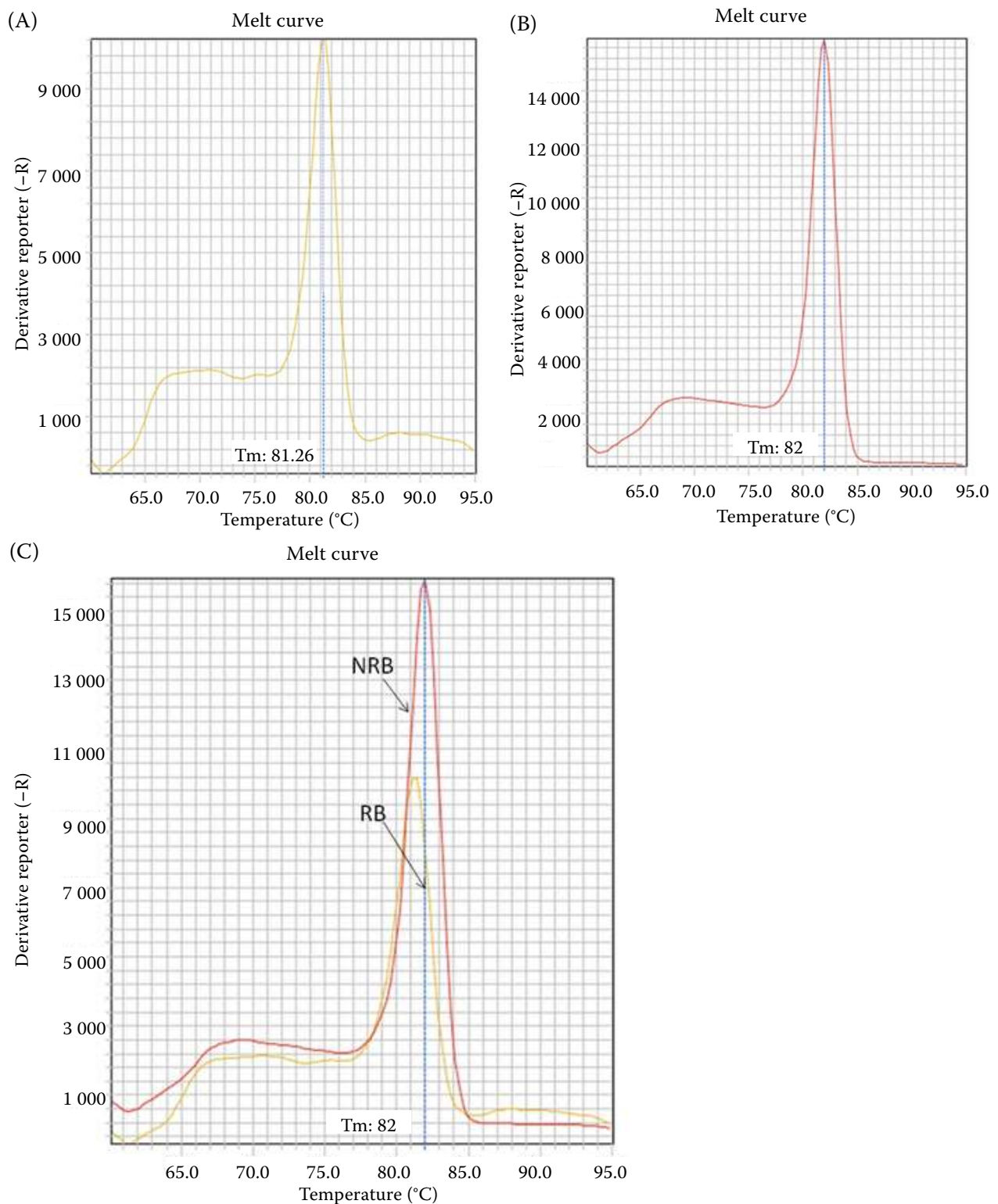


Figure 4. Peak appearance of two isolates at two different temperature values in the melt-curve stage

(A) the isolate that did not break the resistance peaked at 82.0 °C, (B) the isolate that broke the resistance peaked at 81.4 °C, (C) a comparison of two isolates

as having a wide variety of hosts in our country and is an important pathogen causing economic loss in

these crops. This RNA virus is a member of the genus *Orthospovirus*, part of the *Tospoviridae* fam-



Figure 5. The mutation was detected at point C118 in isolates collected from surveyed areas. Substitution of adenine for guanine. Point mutation is highlighted in green

ily and the order *Bunyavirales*, and is propagatively transmitted by thrips. This study explores the significance of TSWV in the disease cycle, infection status, and host positioning during both summer and winter weed growth, transmitted by thrips.

In the majority of recorded cases of TSWV infection in weeds, it was discovered that infected weeds do not exhibit apparent symptoms, and TSWV infection rates in weeds were also reported to be low (Batuman et al. 2020). As a result, whether symptoms are missing or present in weeds, they are not reliable visual indications of TSWV infection. However, it has been noted that existing symptoms may not be caused by TSWV but by other abiotic or biotic causes. ELISA results revealed lower absorbance values, indicating that they fell within the range considered negative in the evaluation (i.e., their values were not three times higher than the negative reference) based on absorbance values at 405 nm. This was observed even though the plants exhibited some leaf lesions that could be associated with TSWV. The current study noted that several weeds infected with TSWV remained asymptomatic or were not related to the symptoms, which had previously been described in various species and other viruses (Morca et al. 2022).

Non-structural protein (NSm protein) is a protein that facilitates the spread of Orthotospoviruses in plants through the cell-to-cell movement of non-enveloped nucleocapsids via plasmodesmata (Lewandowski & Adkins 2005). Previous studies have reported that mutations in the C118Y point on the

NSm protein in the medium segment are key to the adaptation of TSWV on new hosts (Lopez et al. 2011). In line with the sequence data obtained from the infected weeds in this study (Figure 5), it was discovered that the RB isolates emerged due to the point mutation on the NSm-movement protein in the medium segment of the virus (Batuman et al. 2017; Fidan & Sari 2019; Ferrand et al. 2019). To determine TSWV isolate in any study, the status of TSWV can be easily understood through optimisation and verifications. As such, temperature values are less used at the melting curve stage (Lahre et al. 2023).

TSWV-infected tomato and pepper samples were also collected in greenhouses to test Koch's hypotheses about whether the non-RB isolate could infect resistant plants. Through molecular investigations, it was determined that the infection observed in these samples resulted from RB isolates. This indicates that thrips vectors transmitted the TSWV-RB isolate from naturally infected resistant plants.

It is widely recognised that the climatic circumstances of the disease's location, in the presence of vectors and hosts, are essential in analysing the virus's heredity and determining the prevalence of mutations (Nachappa et al. 2020). It has also been noted that weeds (both perennial and annual) in and around the greenhouse serve as significant hosts for thrips' survival and reproduction. As a TSWV inoculum source, this condition poses a significant concern. In other words, TSWV-infected weeds serve as the first and natural hosts for TSWV to overwinter and spread via insect vectors. *Cheno-*

podium album samples with no visible symptoms were found to be virus-free after numerous previous observations in Türkiye, and none of them were found to be TSWV positive. Research of symptomatic tomatoes, peppers, and weeds in five different provinces of Türkiye found that TSWV infection in *C. album* was uncommon when compared to other weed hosts. This was the first time TSWV was found in *C. album* in Türkiye, demonstrating weed species' epidemiological function as virus reservoir hosts (Morca et al. 2022). In the current investigation, a perennial weed, *C. arvense* (creeping thistle), was sampled soon after it emerged from the soil in the spring season and was shown to be infected with TSWV (50%). *C. arvense* is a weed that reproduces by rhizomes and seeds, and given the thrip's life cycle, this species can always be a source of infection due to its perennial and vegetative nature. Furthermore, each growth stage of such plants is significant in understanding TSWV dissemination and epidemiology. As a result, plant leftovers contaminated with vectors should be removed in order to reduce or eliminate the virus's inoculum source. Soil cultivation and weed control should be achieved satisfactorily, and ventilation apertures should be covered with tulle (462 m).

TSWV's summer weed hosts

The results obtained from the present studies revealed that 42% of the collected weeds belong to the *Asteraceae* family, 10% to the *Poaceae* family and 48% to other infected species and 11 infected species were weeds found during the summer season, and eight infected weeds were from the winter season. Among the annual summer weeds, *S. nigrum* (Black nightshade) showed the highest infection rate (3.742 absorbance at 405 nm) and was the most common weed species infected with this virus in coastland and highland locations. The infection rate in the sampled weeds was found to be higher than the other reports in previous years (Atakan et al. 2013; Macharia et al. 2016). Moreover, it has been observed that symptoms associated with TSWV were more pronounced in greenhouse settings than in field conditions. It has been noted that virus symptoms are notably more intense than usual, particularly in greenhouses cultivating various plants like parsley, spinach, onion, eggplant, chard, and beans for fresh consumption. This is especially evident near areas where tomatoes and peppers are grown.

S. halepense and *D. sanguinalis* were among the monocotyledonous summer weeds that were

found to be infected with TSWV. Although these two weeds are not common hosts of TSWV they were found to be positive for TSWV infection, revealing the importance of these host species. Among the other annual summer weeds collected, the estimated infection rates were 25% in *C. canadensis*, 26% in *S. nigrum*, and 50% in *X. strumarium*. In addition, TSWV infections were detected in *L. serriola*, *A. chlorostachys*, *P. oleracea* and *T. terrestris*.

TSWV's winter weed hosts

During the present studies, the TSWV infection was found in winter weeds like *V. hederifolia*, *C. arvensis*, *Medicago sp.*, *Raphanus raphanistrum*, and *P. aviculare*.

The evaluation of the life cycle of natural hosts of TSWV in the present study confirmed that nine host species showing positive results for viral infection were winter-season plants (47%), and ten host species were summer-season hosts (53%). Since most of these species overwinter in heated greenhouses in TSWV areas, almost the same proportion of winter weeds have been confirmed positive compared to summer weeds. Previous research has shown high rates of TSWV infection in *Gamochaeta falcata* (L) Cabrera, *Stellaria media* L., *Lepidium virginicum* L., *Geranium carolinianum* L., and other weeds. Similarly, a high percentage (up to 20%) of some winter weed species has been reported to be infected with TSWV (Groves et al. 2001; Mullis et al. 2009).

The results from the survey studies showed that many TSWV-infected winter weed species (*S. vulgaris*, *C. arvense*, *S. asper*, and *S. oleraceus*) matures with the emergence of many TSWV-infected summer species (*X. strumarium*, *C. canadensis*, and *A. chlorostachys*). These cottage weeds appear to be infected early in their development and harbour the TSWV source throughout the summer. During the germination of winter weed species with high susceptibility to viral infection, many diseased plants of the same species were encountered in autumn surveys. *S. asper* (prickly sow-thistle) showed the highest infectivity among the annual winter weeds. It was also determined that it emerged as a dominant species in greenhouses in the coastland location in winter. These results suggest that both winter and summer weeds play an important role in the absence of the main host for TSWV.

CONCLUSION

TSWV-RB strain is recognised for affecting tomatoes carrying the *Sw-5* gene and peppers with the *Tsw* gene. This particular isolate is widespread in Antalya, Türkiye, a region renowned for greenhouse cultivation. Regarding the changes in virulence of the TSWV isolate, which has been a problem in Antalya province due to increasing air temperatures, it was considered that this circumstance contributed to the emergence of the isolate that broke both temperature and resistance owing to mutation. Infected weeds and ornamental plants also serve as potential hosts for this virus. As a result of ineffective weed control, they constitute a source of transmission point for TSWV vectors. Based on this information, it is critical to minimise the quantity of weeds in tomato and pepper-producing regions as much as possible, particularly in greenhouses, to control the development of TSWV. However, more research on the interactions between weeds as thrips and other insect vectors and virus sources is needed to understand their effects on TSWV survival and dissemination and avoid large crop losses.

CONFLICT OF INTEREST

The authors declare that they do not have any known competing financial interests or personal ties that could appear to have influenced the work disclosed in this study.

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