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Research Article

New insights into tomato spotted wilt orthotospovirus (TSWV) infections in Türkiye: Molecular detection, phylogenetic analysis, and *in silico* docking study

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Abstract

Seventy-eight tomato and pepper plants showing symptoms were tested for tomato spotted wilt orthotospovirus (TSWV) using specific primers targeting the full coat protein gene (CP) through RT-PCR. Plant samples were collected in Denizli region, Türkiye during September 2021. The PCR screening results revealed that 23 samples were infected with TSWV, indicating an infection rate of 29.48%. PCR products were subsequently sequenced bidirectionally, and the obtained sequences were deposited in GenBank under the accession numbers ON323583-84 for pepper, and OQ597214 for tomato. Phylogenetic analysis demonstrated a close relationship between the Turkish-Denizli TSWV isolates and previously reported isolates from tomato and pepper plants in Türkiye (KM407603), Hungary (KJ649612), and Serbia (GU369723). Moreover, in this study, three commercial chemicals and five selected phytochemicals were docked against the TSWV CP to assess their binding energies. The docking results indicate that the tested phytochemicals exhibit promising performance compared to other commercial chemicals. This study represents the first molecular and phylogenetic report on TSWV isolates in pepper and tomato plants in Denizli province, Türkiye. Furthermore, the interaction between TSWV and the selected compounds through *in silico* docking has been reported for the first time.

Keywords: *in silico* modelling; molecular docking; phylogeny; RT-PCR; TSWV

Introduction

Biotic agents that infect tomato and pepper plants pose significant challenges to sustainable vegetable agriculture worldwide. Among these agents, obligate parasitic plant viruses have a substantial impact on crop

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quality and yield, leading to annual losses worth billions of dollars (Mumford *et al*., 2016). The interaction between the host and virus proteins plays a crucial role in disrupting plant cell homeostasis. This interaction results in various effects such as impaired integrity of intracellular membranes, altered rates of photosynthesis and respiration, imbalanced distribution of carbohydrates among plant tissues, and dysfunctional protein metabolism in the host (Aranda and Maule, 1998; Stange, 2006; Laliberté and Zheng, 2014; Ertunç, 2020).

Tomato spotted wilt orthotospovirus (TSWV) is considered one of the top 10 viruses worldwide that affect crop production, ranking second after tobacco mosaic virus (TMV) (Scholthof *et al*., 2011). The diameter of the TSWV particle, which is roughly spherical, ranges between 80 and 110 nm. It is composed of 5% nucleic acid (RNA), 70% protein, 20% lipid, and 5% carbohydrate (Adkins, 2000). The virion is enveloped with glycoproteins and consists of three single-stranded linear RNAs, named L, M, and S, in decreasing order of size. Each of the RNAs has a different sense polarity, with one being negative and the other two ambisense (Adkins, 2000; Tsompana and Moyer, 2008).

TSWV was first identified in Australia in 1915 and has since been reported in numerous other countries (Brittlebank, 1919). Belonging to the Bunyaviridae family and the genus *Orthotospovirus*, TSWV has a broad host range, infecting more than 925 plant species, including cultivated crops, ornamental plants, and weeds. It can support roughly ten thrips species that act as vectors for transmission (Pataky, 1991; Sherman *et al*., 1998; Arli-Sokmen *et al*., 2005; Pappu, 2008). TSWV causes significant losses in various crops, such as tomatoes, peppers, potatoes, eggplants, lettuces, beans, artichokes, celery, and tobacco (Rosello *et al*., 1996; Abad *et al*., 2005).

Agricultural pests, particularly certain thrips species such as *Frankliniella occidentalis*, play a significant role in the transmission of viral diseases, including mechanically transmitted and non-seed-borne viruses. TSWV is transmitted by thrips via circulative and propagative mechanisms (Goldbach and Peters, 1994; Pappu, 2008). TSWV may replicate its genome within thrips, indicating that thrips are important mobile hosts for the virus (Ullman *et al*., 1993; Goldbach and Peters, 1994).

TSWV infection in plants leads to various symptoms, including the formation of necrotic, chlorotic, and reddish rings, mosaic patterns, tanning, leaf curling, and mottling. In the early stages of infection, wilt-induced drooping leaves, unilateral growth failure, stunting, and even death of young plants can occur. As the fruit matures, later infections result in the development of unmarketable fruits characterized by striking chlorotic/necrotic ring spots and malformed shapes (Chiemsombat and Adkins, 2006).

TSWV has been extensively documented in various plants and plantations (Pappu *et al*., 2009). Examples of its presence include tobacco in Poland and the USA (Jankowski *et al*., 1980; Mandal *et al*., 2008), tomatoes and peppers in the USA, Canada, and Kenya (Gitaitis *et al*., 1998; Wangai *et al*., 2001), lettuce in Australia-Tasmania (Wilson, 1998), and tomatoes, peppers, tobacco, and peanuts in the USA-Georgia (Wells *et al*., 2002). The presence of TSWV has also been well-studied in many agricultural areas in Türkiye. The virus was first reported in lettuce plants by Tekinel *et al*. (1969) and has since been detected at varying rates in tomatoes (Arlı-Sökmen and Sevik, 2006), tobacco (Azeri, 1994), pepper, zucchini (Yardimci and Kilic, 2009), and eggplant (Kamberoğlu *et al*., 2009). The majority of studies in Türkiye have relied mostly on serological approaches, with only a few molecular-based investigations (Morca *et al*., 2022). The objective of the present investigation was to conduct a molecular analysis for the purpose of detecting tomato spotted wilt orthotospovirus (TSWV) infection in tomato and pepper plants grown in the Denizli province of Türkiye, as well as to analyze its phylogenetic relationships.

For a significant period, viral infections have posed a threat to agricultural sustainability (Hari and Das, 1998; Karen-Beth *et al*., 2011). Researchers have dedicated considerable effort over the past few decades to find effective methods of controlling these infections (Wang *et al*., 2012; Yang *et al*., 2010). Biological and chemical approaches are being explored with optimism in this regard. Nucleoside analogs, known as pro-drugs, have shown promise in exhibiting antiviral activity by converting into nucleotide metabolites. These analogs must undergo conversion into nucleotides by host enzymes contained in pyrimidine and purine metabolism to prevent viral replication. The selectivity of nucleoside analogs is based on their ability to block viral target enzymes, as this activation into nucleotides occurs in both the virus and the host cell. Ribavirin, also known as virazole (1-b-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), is an antiviral nucleoside analog with a broad spectrum of antiviral activity (Sidwell *et al*., 1972). Recent research suggests that ribavirin's antiviral effectiveness is due to its capacity to increase the frequency of damaged viral RNA genomes by inducing mutations in viral RNA (Severson *et al*., 2003). However, the precise mechanism of its antiviral activity remains unknown and may vary depending on the specific virus and host (Parker, 2005).

Plants produce compounds through unique metabolic pathways, and these compounds have shown potential in both preventing and treating diseases caused by microorganisms (Demirel *et al*., 2022). Several studies have demonstrated that extracts from medicinal plants can be used as biocontrol agents to manage various plant diseases and can induce plant resistance (Devjani and Sunanda, 2010; Shabana *et al*., 2017; Santamaria-Hernando *et al*., 2019). Multiple factors, including host species and variety, host physiology, age, nutrition, climate, the presence of other viruses, and more, may influence the nature and severity of TSWV symptoms. The interaction between the virus and the host plant is complex and influences the manifestation of symptoms. To better understand the interaction between viral proteins and various ligand molecules, computational approaches such as *in silico* modeling and molecular docking have been employed. These methods are used to evaluate the antiviral activity of commercially available compounds like ribavirin and tunicamycin, as well as phytochemicals such as quercetin and catechin, against TSWV. The study presented in this context aimed to analyze the anti-TSWV potential of these compounds by docking and examining their binding interactions with the CP protein of TSWV. The findings from these computational analyses provide valuable insights that can contribute to the development of novel organic formulations for managing this devastating viral disease in agricultural crops.

Materials and Methods

Sampling and complementary DNA synthesis (cDNA)

A total of 78 leaf samples from-greenhouse-cultivated tomato and pepper plants showing fruit with brown, yellow, or reddish rings and malformed fruits were collected in Denizli province, Türkiye during September 2021 and total nucleic acids were extracted, according to the method reported by Foissac *et al*. (2001). The RNAs were used as templates for reverse-transcription using a random primer according to the kit protocol provided by the company (Thermofisher Scientific, USA).

Polymerase chain reactions (PCRs)

In this study, polymerase chain reactions (PCRs) were performed using cDNAs synthesized from the extracted TNAs as templates. Specific forward and reverse primer pairs were designed to amplify a 777 bp fragment of the whole CP gene of TSWV. The forward primer sequence was TSWV-F-5'-ATG TTT AAG GTT AAG CTC AC-3', and the reverse primer sequence was TSWV-R-5'-TTA AGC AAG TTC TGT GAG TT-3'. The thermocycling conditions for the PCR amplifications consisted of an initial denaturation step at 94 °C for a duration of 2 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 53 °C for 30 seconds, and extension at 72 °C for 2 minutes. A final extension step was performed at 72 °C for 10 minutes. The PCR products, along with a positive control, negative control, and a 1kb DNA marker (Fermentas, Lithuania), were visualized on a 1% agarose gel stained with ethidium bromide and monitored under UV light (Syngene™, UK). The PCR reaction mixtures consisted of 25 µl, which contained 19.3 µl of nuclease-free water, 2.5 µl of 10X PCR buffer, 1.5 µl of $MgCl_2$ (25 mM), 0.5 µl of dNTP (10 mM), 0.5 µl of each forward and reverse primer (20 pmol), and 0.2 µl of Taq DNA polymerase enzyme (5U/µl) (Thermofisher

Scientific, USA). A pre-sequenced TSWV isolate was used as the positive control, and healthy plants served as the negative control in the tests.

Sequencing and phylogenetic relationships

In this study, three PCR-positive products, two from pepper and one from tomato, were selected for further analysis. These products were purified from the agarose gel using a purification kit from Thermo Fisher Scientific (USA). The purified PCR products were then sent to BM Labosis (Ankara, Türkiye) for sequencing. The provided sequences were validated by performing a BLAST analysis on the NCBI database to confirm their similarity to known sequences, and registered in GenBank. To compare the phylogenetic relationships of these sequences to those of other isolates from around the world, the RNA S gene segment of approximately 2900 base pairs in length, which contains the sequence encoding the nucleocapsid protein, was included in the phylogenetic tree. The phylogenetic dendrogram was constructed using the Neighbor-joining algorithm and Tamura-3 parameter with 1000 bootstrap replicates in Mega11 software.

In silico analysis and molecular docking of CP

The EXpasy Translate program was used to convert the coat protein (CP) gene sequences of the three TSWV isolates obtained in the present study into amino acid sequences. (Gastiger *et al*., 2003). The physicochemical characterization of the amino acid sequences of the CP genes was conducted using Expasy's ProtParam server (http://web.expasy.org/protparam/), which allows for the computation of various protein properties. Multiple alignment of the protein sequences was performed to identify conserved regions, and homology modeling of the TSWV coat protein was carried out using the I-TASSER, Swiss-Model and Phyre2 web servers. The secondary structure of the TSWV CP was predicted using the SOPMA server (http://npsapbil.ibcp.fr/). The resulting 3D structures in PDB format were submitted to PROCHECK in the Structural Analysis and Verification (SAVES 6.0) platform (https://saves.mbi.ucla.edu/) for further analysis. The stereochemical validity of the model was verified using the Ramachandran plot generated by PROCHECK (Laskowski *et al*., 1993) to determine which percentages of the model's residues were in favored and disallowed regions.

The UCSF Chimera software was used to generate PDBQT files for the receptors and ligands. The study conducted by Gowthaman *et al*. (2008) utilized a semi-flexible docking approach wherein the receptors were maintained in a rigid state while the ligands were permitted to exhibit flexibility. The protein receptors were generated through the elimination of water molecules and hydrogen bonds, combining of non-polar atoms, and the assigning of Kollman and Gasteiger charges. The 3-dimensional structures of all ligand molecules were acquired from the PubChem database of ligands (https://pubchem.ncbi.nlm.nih.gov/). Using UCSF Chimera, the docking of the target protein CP (NCBI accession: ON323583) with the ligands used in this study was prepared, executed, and analyzed. After preparing the receptor (one subunit of TSWV CP) and the ligand, the AutoDock Vina tool within UCSF Chimera was utilized to perform molecular docking analysis, assessing hydrogen bond interactions and binding affinities.

Results

Symptomatology and viral detection

In our study conducted in the Denizli province of Türkiye in 2021, we performed PCR-based molecular methods to identify TSWV isolates in symptomatic tomato and pepper samples. The observed symptoms included reddish/light yellow circular lesions on the fruits, white concentric rings on the leaves of peppers, and general wilting, bronzing, and necrosis on the leaves of tomato plants (Figure 1).

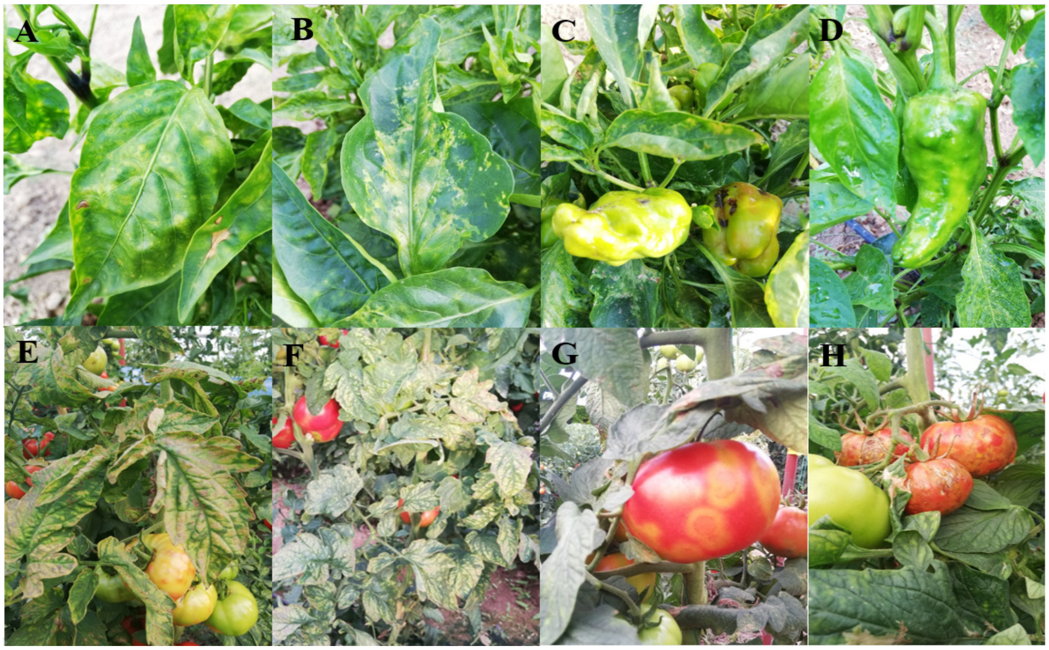


Figure 1. Symptoms triggered on fruit and leaves by TSWV infection in tomato and pepper plants from Denizli province, Türkiye. (A, B) Light colored annular spots, chlorotic spots, and disfigurement on pepper leaves; (C, D) necrotic lesions on fruit and deformed fruit; (E, F) bronzing and severe chlorotic areas on the leaves; (G, H) yellow ring spot, sunken necrosis areas, and malformation in tomato fruit

In this study, out of 78 leaf samples collected from Denizli province, 23 tested positive (10 pepper and 13 tomato) for TSWV in PCR tests. The RT-PCR generated an amplified product of 777 bp, which corresponds to the TSWV-CP gene in infected samples. No DNA fragments were detected in the negative control, which consisted of healthy plants. Two peppers and one tomato isolates, which were randomly selected positive samples, were re-amplified using PCR to obtain sufficient DNA concentration. The sequences of the purified fragments that were directly sequenced.

Sequencing and nucleotide similarity

The full-length CP gene sequences consisting of 777 nucleotides were recorded into GenBank with accession numbers ON323583-84 for pepper and OQ597214 for tomato. The BLAST analysis of Denizli TSWV sequences revealed nucleotide sequence similarities ranging from 97.17% to 98.58% with TSWV sequences from different hosts and regions worldwide.

The sequences were then compared to TSWV isolates from various hosts and agroecological zones to determine the nucleotide identity index. The analysis based on the Sequence Demarcation Tool revealed that all isolates exhibited sequence homology ranging from 95% to 100%. Denizli isolates exhibited approximately 99% nucleotide similarity, which is consistent with the molecular phylogeny (Figure 2). Furthermore, the nucleotide consensus among all isolates in the group, including the Denizli TSWV isolates, was calculated to be over 98%.

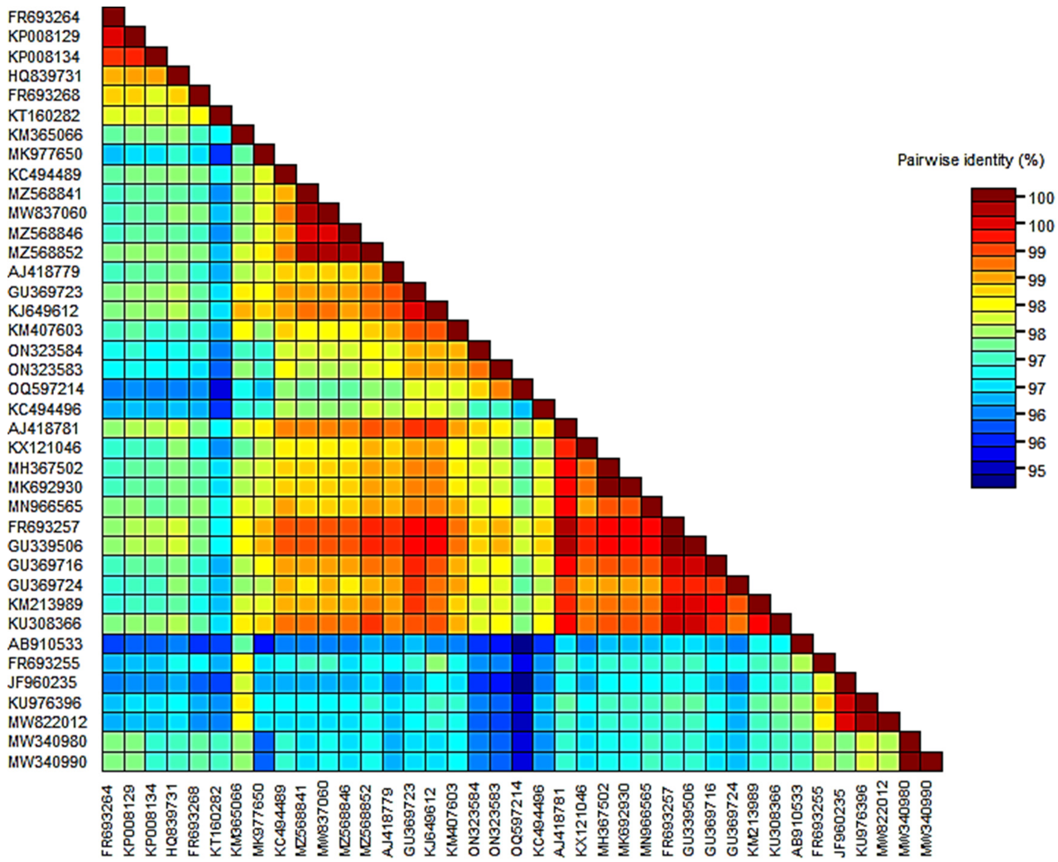


Figure 2. Nucleotide sequence similarity matrix constructed based on the full coat protein gene using the TSWV isolates identified in this study and other TSWV isolates from around the world

Molecular phylogeny of Denizli-TSWV isolates

A phylogenetic tree was generated utilizing the Mega11 software, incorporating the isolates employed in the present investigation and 36 other isolates obtained from GenBank (Table 1). The tree classified TSWV isolates into main two groups, based on nucleotide homogeneity. As illustrated in Figure 3, TSWV-Denizli isolates were categorized together with isolates previously discovered on tomato and pepper isolates from Türkiye (KM407603), Hungary (KJ649612), and Serbia (GU369723). The analysis of the genetic relationships in this group revealed that TSWV isolates formed a distinct cluster based on their host specificity. However, when other groups like the group including our isolates were investigated, a mixed collection of isolates obtained from diverse plants including tomatoes, peppers, tobacco, *Lysimachia* sp., *Stellaria media*, and *Calceolaria* sp. across several countries such as Italy, Pakistan, Türkiye, France, Montenegro, New Zealand, Germany, and Serbia were observed to cluster together. Surprisingly, TSWV isolates from other Turkish regions, including Konya, Ankara, and Eskişehir provinces, were included in the third group (pink) along with isolates from New Zealand and India, independently of the TSWV isolates from Denizli province. In conclusion, it can be generally stated that the clustering of TSWV isolates in the same group is not dependent on geographic origin or plant source.

Table 1. Information regarding the isolates identified in various hosts and ecological areas employed to construct the phylogenetic tree

No	Isolate name	Host	Country	Accession no	Size (bp)
1	AT1	*Brugmansia sp.*	South Korea	AB910533	777
2	SO-Tomato	*Solanum lycopersicum*	South Korea	MW340990	777
3	SO-Pepper	*Capsicum annuum*	South Korea	MW340980	777
4	TSWV-LNSY	Pepper	China	MW822012	777
5	TSWV-YN	Tomato	China	JF960235	2970
6	TSWV-LE	Lettuce	China	KU976396	2970
7	DH-37	Tomato greenhouse	Bulgaria	AJ418779	2948
8	p105-RB-MaxII	Pepper	Italy	HQ839731	2927
9	p331	Pepper	Italy	KM213989	777
10	AAICPK	Capsicum	Pakistan	KX121046	777
11	TSWV-PK1	Tomato	Pakistan	MN966565	777
12	Ka-To	Tomato	India	MK977650	2948
13	PA01	Pepper	USA	KT160282	2984
14	TomUSA	*Solanum lycopersicum*	USA	FR693268	774
15	PVR	Pepper	Spain	KP008134	2951
16	LL-N.05	Tomato	Spain	KP008129	2924
17	T3-2	*Solanum lycopersicum*	Spain	FR693264	774
18	WA7	*Solanum lycopersicum*	Australia	KM365066	2899
19	STM3B	*Stellaria media*	France	FR693257	774
20	SO46	*Solanum lycopersicum*	France	FR693255	774
21	Is-141	*Calceolaria sp.*	Montenegro	GU339506	777
22	Is-334	*Nicotiana tabacum*	Montenegro	GU369716	777
23	PFR07	*Solanum lycopersicum*	New Zealand	KC494496	777
24	MAF11	*Ocimum basilicum*	New Zealand	KC494489	777
25	TW-307	*Solanum lycopersicum*	Türkiye (Ankara)	MZ568841	777
26	TW-432	*Solanum lycopersicum*	Türkiye (Eskisehir)	MZ568846	777
27	TRpep-125/6	*Capsicum annuum*	Türkiye	MW837060	777
28	Antalya	*Capsicum annuum*	Türkiye (Antalya)	KM407603	777
29	TW-581	*Solanum lycopersicum*	Türkiye (Konya)	MZ568852	777
30	TSWVAntRB	Tomato	Türkiye	MH367502	2961
31	TswvTRPep	*Capsicum annuum*	Türkiye	MK692930	2961
32	HUP5-2009-WT	Pepper	Hungary	KJ649612	2948
33	B	*Ocimum basilicum*	Poland	KU308366	777
34	LE98/527	*Lysimachia sp.*	Germany	AJ418781	2965
35	Sr-739	*Nicotiana tabacum*	Serbia	GU369724	777
36	Sr-603	*Lycopersicon esculentum*	Serbia	GU369723	777

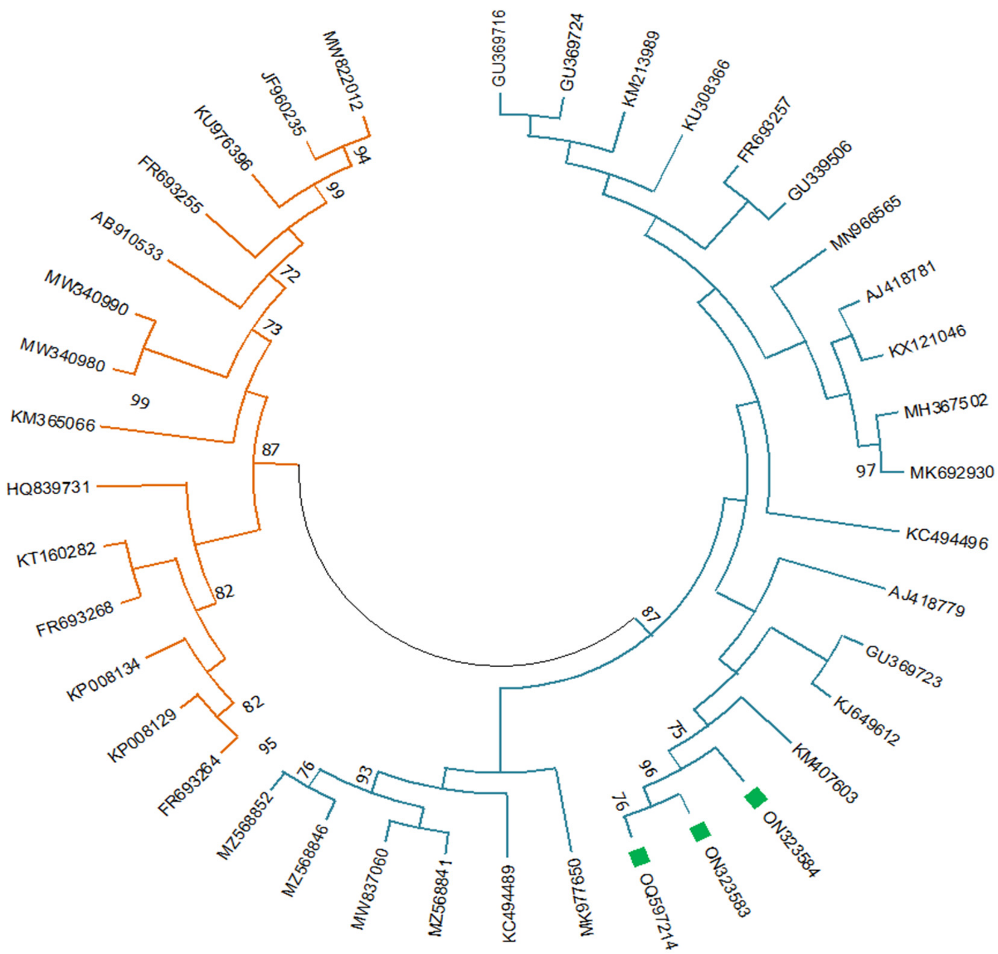


Figure 3. The cladistic phylogram created by the Neighbor-Joining Method showing the phylogenetic relationship of tomato spotted wilt orthotospovirus from pepper and tomato plants of Denizli province of Türkiye. The Denizli isolates (ON323584, ON323583, OQ597214) are located in the green-colored box section.

In silico and docking analysis

The physicochemical characterization of CP proteins of Denizli TSWV isolates was performed and was presented in Table 2. Among the calculated physiochemical properties, all except for EC and II exhibited similar values. A comparison analysis revealed that the II values of CP proteins are less than 40. According to Guruprasad *et al*. (1990), a protein is considered stable if its II value is below 40, whereas any value above 40 indicates protein instability. CP proteins of TSWV isolates are stable according to II index. Additionally, the GRAVY score range of -0.147 to -0.169 for the CP proteins of Denizli TSWV isolates indicates that the overall protein is hydrophilic and soluble.

Table 2. Coat protein's physical and chemical traits by Expasy's Protparam tool

Physicochemical parameters	ON323583	OQ597214
Naa	258	258
PI	9.19	9.19
M.Wt (Da)	28932.81	28932.75
EC	10555	16055
-R	31	31
+R	39	39
II	33.40	32.20
GRAVY	-0.147	-0.156
AI	96.01	95.27

SOPMA (Self Optimized Prediction Method and Alignment) was utilized to predict the secondary structures of two proteins. The findings indicated a predominance of alpha helix in the TSWV CPs, as shown in Table 3. The analysis of various conformational states of secondary structures revealed that helices were the most prevalent in the CP of ON323583 (50.00%) and CP of OQ597214 (47.29%) proteins, respectively.

Table 3. Characterization of the coat protein's physiochemical properties using SOPMA prediction algorithm

Parameters	ON323583	OQ597214
Alpha helix (Hh)	50.00%	47.29%
Random coil	26.36%	28.68%
Pi helix (Ii)	0.00%	0.00%
310 helix (Gg)	0.00%	0.00%
Extended Strand (Ee)	17.05%	17.05%
Beta Turn (Tt)	6.59%	6.98%
Bend region (ss)	0.00%	0.00%
Ambiguous states	0.00%	0.00%
Other states	0.00%	0.00%
Beta bridge (Bb)	0.00%	0.00%

Protein models of the CP of TSWV Denizli isolates were generated using the Phyre2 server. Phyre2 server revealed a 95% similarity between the CP proteins of two TSWV Denizli isolates and the c5ip1A template, with a confidence level of 100%. The generated Protein Data Bank (PDB) structures for the CP of the three TSWV isolates are depicted in Figure 4a. Additionally, Table 4 presents the results of the calculations conducted by the Swiss Modeler online tool for various parameters of each gene. The protein structures created by Swiss Modeler can be observed in Figure 4b.

Table 4. Swiss-Modeler's predictions for CP protein model parameters

Parameters calculated via Swiss Modeler	ON323583	OQ597214
QMean	-0.55	0.42
All atom	0.97	1.02
Torsion	-1.27	-1.18
Solvation	1.89	1.89
Cß	0.61	0.83

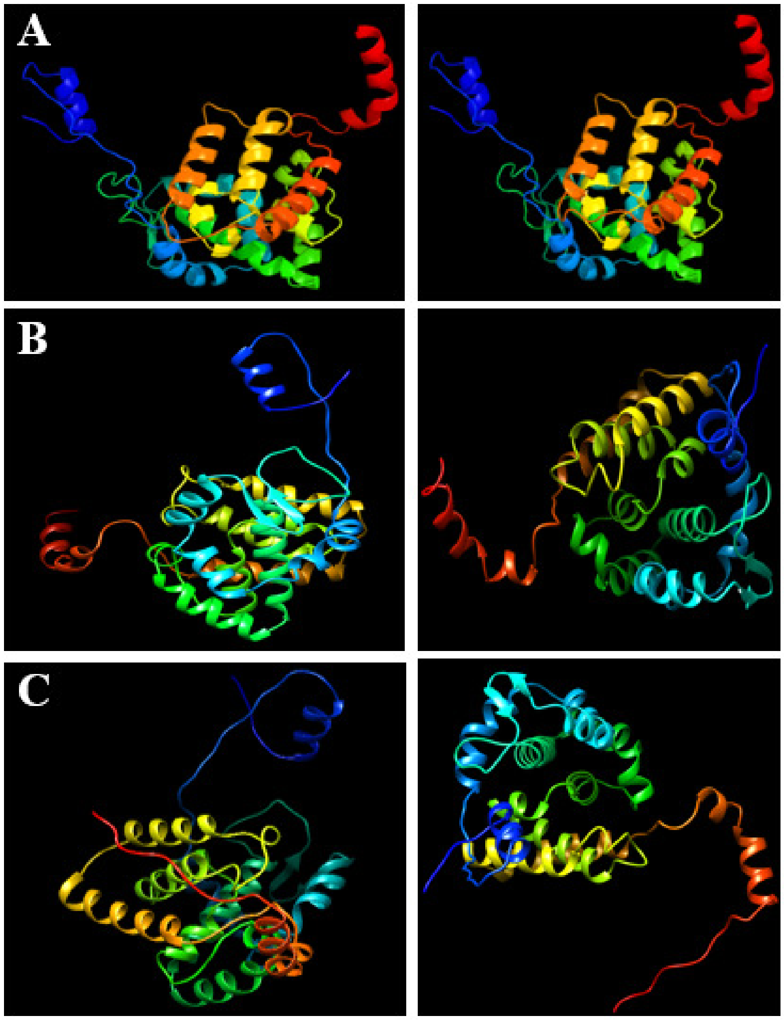


Figure 4. Homology-based modeling belongs to one subunit of CP proteins' 3D structures of ON323583 (in left) and OQ597214 (in right) TSWV isolates. a; Models constructed by Phyre2. b; Models constructed by Swiss-Model. c; Models constructed by I-TASSER

Using the most optimal PDB template structures, the automated comparative protein structure modeling tool I-TASSER identified the templates for one or more sequences. Table 5 and Figure 4c detail the model parameters calculated for each protein model.

Table 5. Parameters of protein models retrieved from I-TASSER

Parameters calculated via I-TASSER	ON323583	OQ597214
Identity	0.95	0.95
C-Score	1.51	1.50
Estimated T-score	0.92±0.06	0.92±0.06
Estimated RMSD	2.9±2.1Å	2.9±2.1Å
Ligand	Nuc. acid	Nuc. acid
Ligand binding sites	32,192	32, 98, 192, 193

The stereo-chemical quality of the final model was assessed using the PROCHECK software of Structural Analysis and Verification (SAVES 6.0) (Laskowski *et al.*, 1993; Sivaramakrishnan *et al.*, 2012; Sidhu *et al.*, 2020), and a Ramachandran plot was generated. Table 6 presents the percentages of residues located in the allowed and not-allowed regions. According to the evaluation of the Ramachandran plot generated by PROCHECK (Figure 5), Swiss-Model reported that 92.2% of the residues were located in the most favored regions, with only 0.1% of the residues in the disallowed areas. Consequently, the TSWV CP produced by Swiss-Model using homology modeling was selected as the target for docking studies.

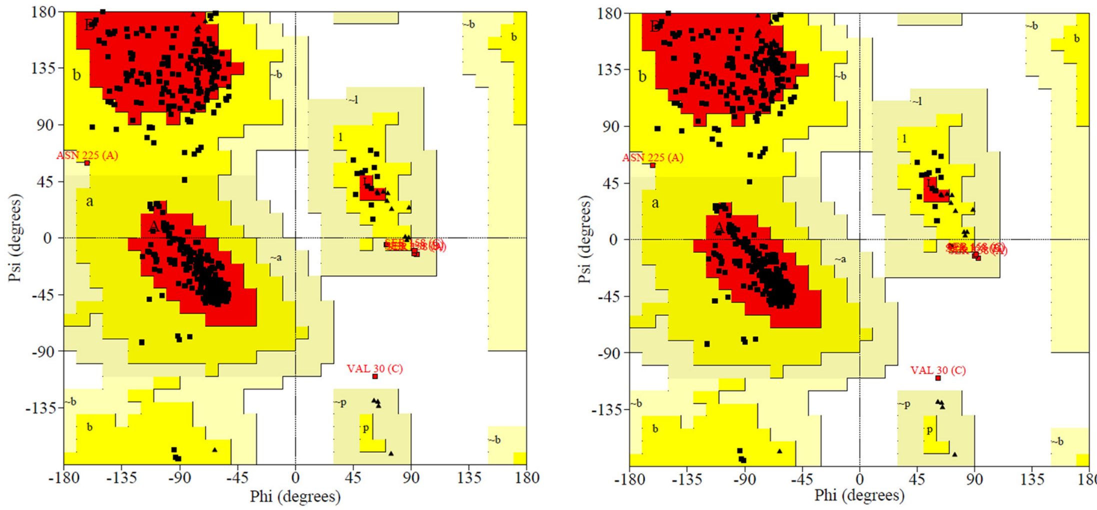


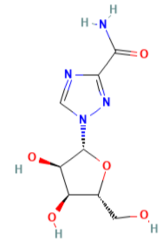
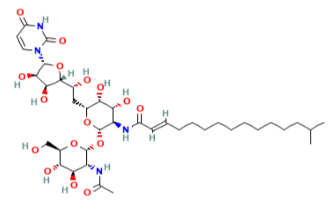
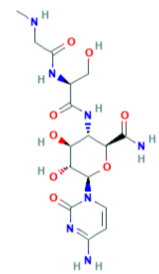
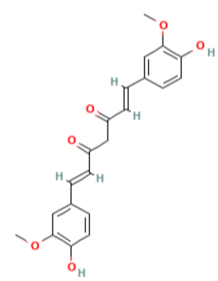
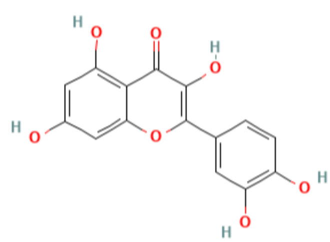
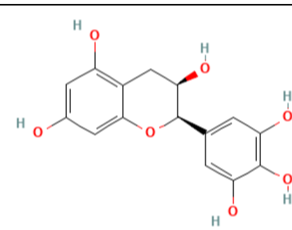
Figure 5. A Ramachandran plot illustrating the TSWV CP homology model (ON323583 on left and OQ597214 on right)

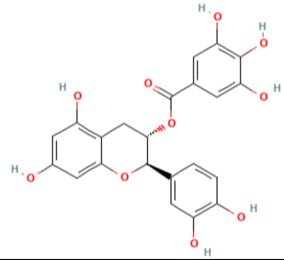
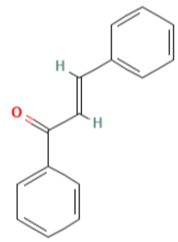
Table 6. Verification of PROCHECK-created models

Proteins (CP)	Phyre model		Swiss model		I-Tasser model	
	PRFR	PRDR	PRFR	PRDR	PRFR	PRDR
N323583	89%	0.4%	92.2%	0.1%	80.8%	1.3%
OQ597214	87.3%	0.9%	92.2%	0.1%	84.5%	0.0%

Table 7 presents the ligand conformations and binding affinities of selected chemicals and phytochemicals that have potential antiviral activity, as reported in the literature. We obtained multiple positions with high binding modes and interconnections within the target pocket, but we selected the ones with the most favorable scores (associated with pose consistency) and RMSD refine values (relative to a specific pose's connection to the initial ligand position within the target pocket). Based on our research findings, the CP of TSWV demonstrated the best docking energy with tunicamycin, which achieved a vina score of -7.6 among the commercially available compounds. This was followed by ningnanmycin with a vina score of -7.0 and ribavirin with a vina score of -5.6. Among the five analyzed phytochemicals, epigallocatechin exhibited the highest docking energy with the CP of TSWV, with a vina score of -8.6. It was followed by catechin (vina score = -7.7), quercetin (vina score = -7.5), curcumin (vina score = -7.1), and chalcone (vina score = -6.8). Figures 6-13 depict visualizations of the interactions between the TSWV CP and the chemicals listed in Table 7.

Table 7. Binding affinities for selected chemicals and phytochemicals

Chemicals/ phytochemicals	PubChem ID	Structure	AutoDock Vina Values
Ribavirin	37542		-5.6
Tunicamycin	56927836		-7.6
Ningnanmycin	44588235		-7.0
Curcumin	969516		-7.1
Quercetin	5280343		-7.5
Epigallocatechin	72277		-8.6

Catechin	5276454		-7.7
Chalcone	637760		-6.8

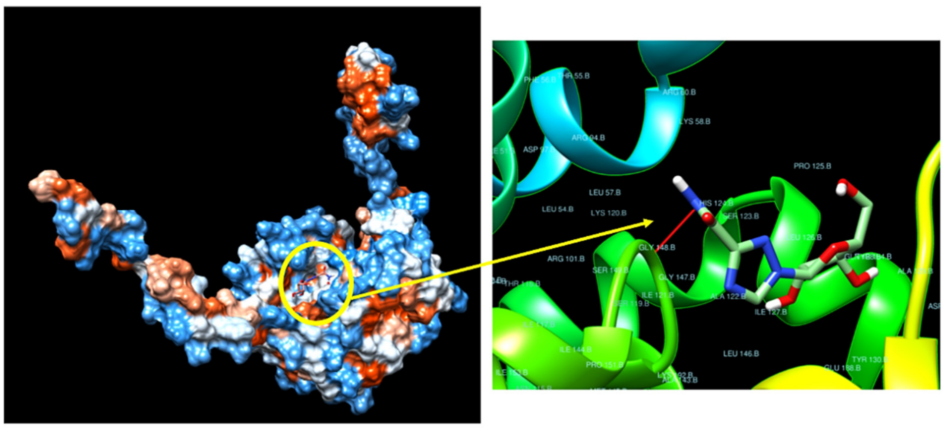


Figure 6. A perspective in three dimensions showing the TSWV CP docking with the ribavirin interacting region. The ligand-binding sites predicted are enlarged

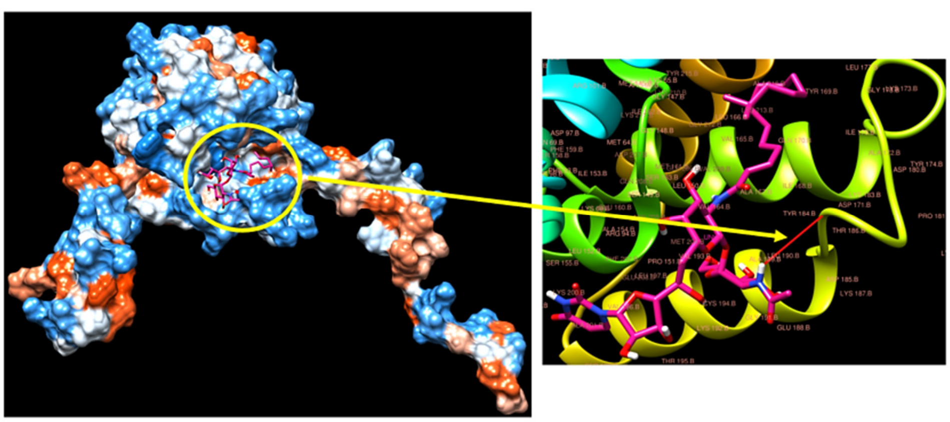


Figure 7. A perspective in three dimensions showing the TSWV CP docking with the tunicamycin interacting region. The ligand-binding sites predicted are enlarged

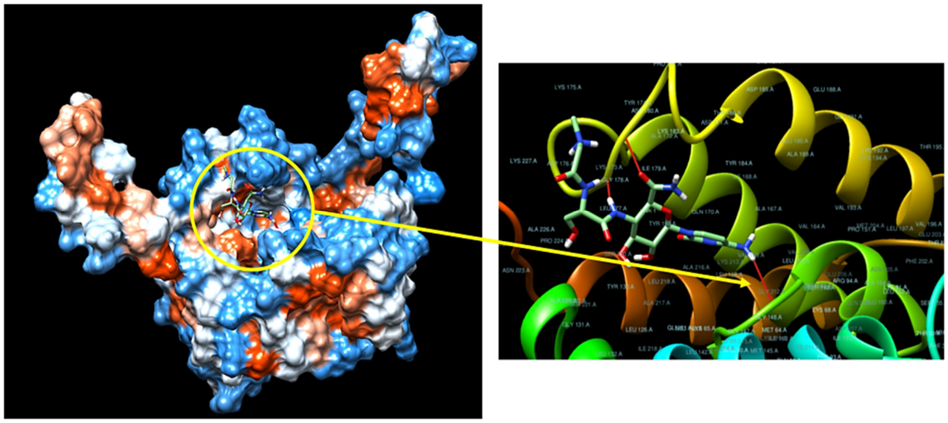


Figure 8. A perspective in three dimensions showing the TSWV CP docking with the ningnanmycin interacting region. The ligand-binding sites predicted are enlarged

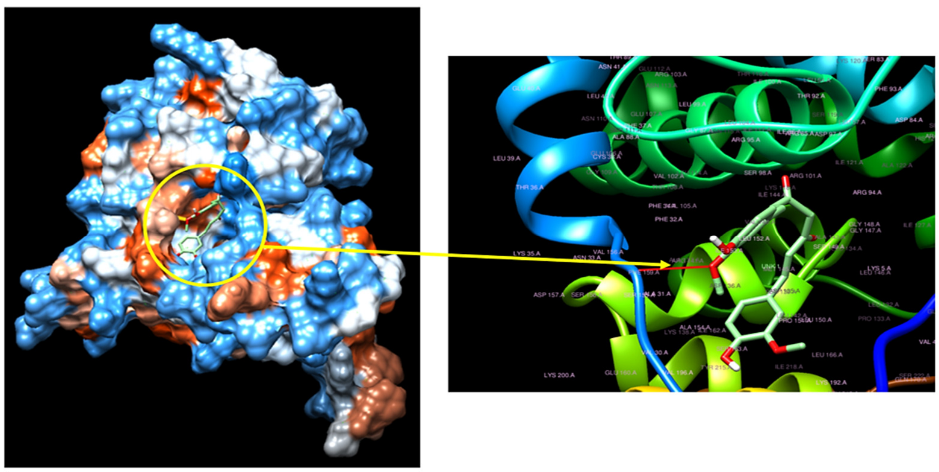


Figure 9. A perspective in three dimensions showing the TSWV CP docking with the curcumin interacting region. The ligand-binding sites predicted are enlarged

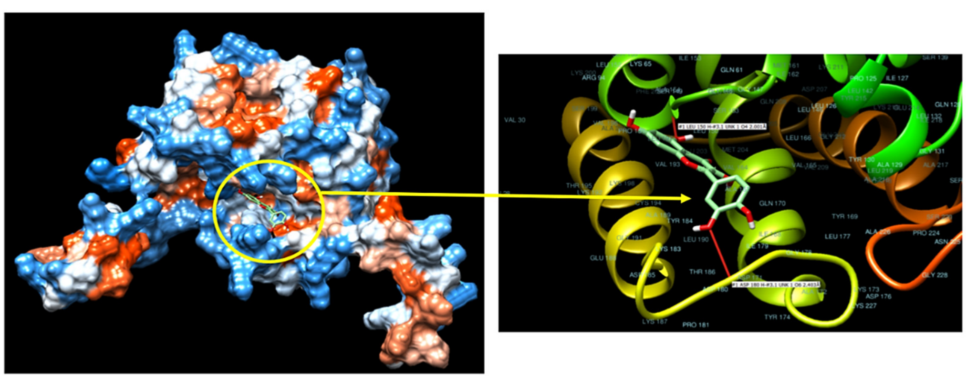


Figure 10. A perspective in three dimensions showing the TSWV CP docking with the quercetin interacting region. The ligand-binding sites predicted are enlarged

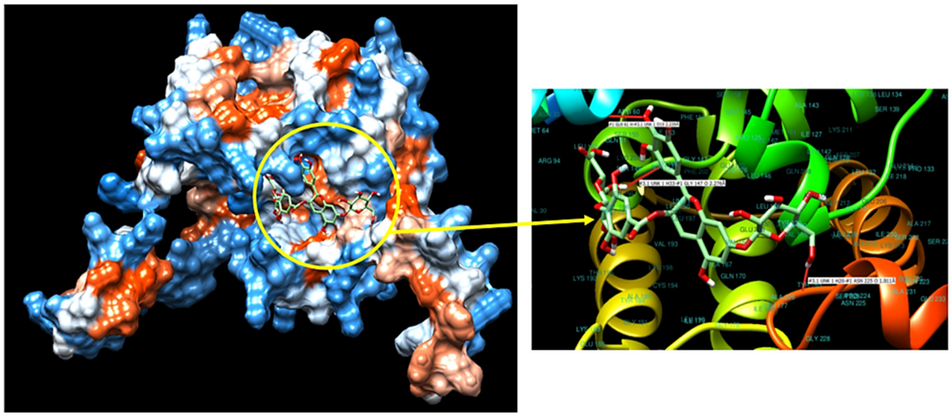


Figure 11. A perspective in three dimensions showing the TSWV CP docking with the epigallocatechin interacting region. The ligand-binding sites predicted are enlarged

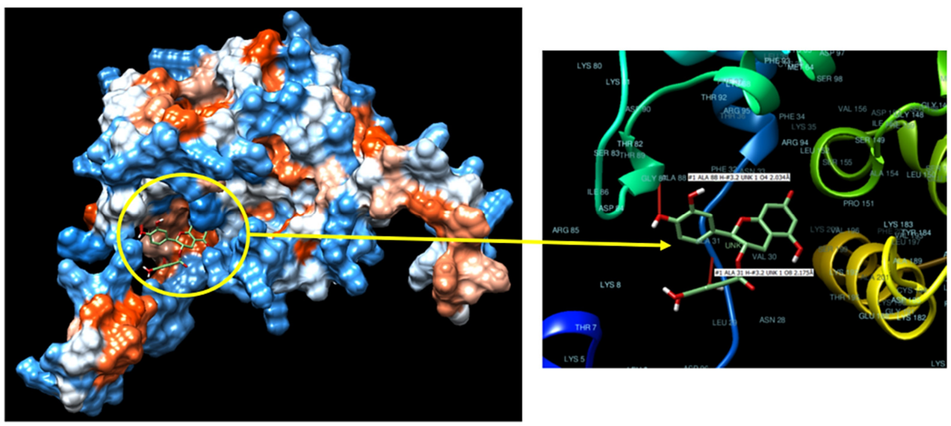


Figure 12. A perspective in three dimensions showing the TSWV CP docking with the catechin interacting region. The ligand-binding sites predicted are enlarged

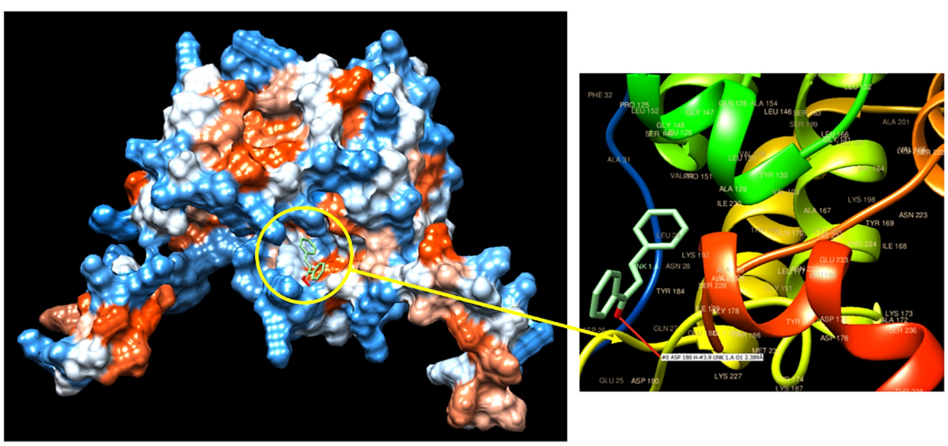


Figure 13. A perspective in three dimensions showing the TSWV CP docking with the chalcone interacting region. The ligand-binding sites predicted are enlarged

Discussion

The symptoms caused by TSWV infection can vary in appearance and intensity depending on numerous factors such as the species and variety of the host plant, the growth stage (e.g., seedling, vegetative, flowering, fruiting), prevailing climatic conditions (temperature, light, etc.), and the specific strain of the virus involved (Adkins, 2000). Consistently, symptoms shown in Figure 1 closely resembled those documented in previous studies conducted in different countries (Zarzyńska-Nowak *et al*., 2018; Morozova *et al*., 2021; Gao and Wu, 2022). TSWV has a wide distribution range across different geographies and hosts worldwide. This virus can cause diseases in many plant species, especially those that are important for agriculture. Since it was initially identified in Australia in 1915, it has spread to numerous locations across the world, particularly in warm and humid conditions. TSWV has been detected in countries throughout Europe, Asia, North America, South America, Africa, and Oceania (Pappu *et al*., 2009). In North America, TSWV has been found in both the United States and Canada, while in South America, it has been reported in Brazil, Mexico, Colombia, Peru, and Ecuador. In Africa, TSWV has been observed in South Africa, Nigeria, and Morocco. Lastly, TSWV has been detected in Australia and New Zealand in Oceania (NCBI, 2023). It is worth noting that this list is not exhaustive as TSWV has been found in many other countries as well.

In recent years, there has been an increasing research focus on TSWV in Türkiye. The presence of TSWV has been reported in solanaceous plants, weeds, and cucurbitaceous plants using serological and molecular methods (Azeri, 1994; Yardimci and Kilic, 2009; Kamberoğlu *et al*., 2009; Topkaya, 2021). Akdura and Culal-Kılıç *et al*. (2022) detected TSWV in 26 out of 184 plants showing signs of yellowing, necrosis, mosaic and deformation in Hakkari province, using the Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA). Serological analysis has revealed the presence of TSWV and CMV in greenhouse peppers in Antalya province, Türkiye. Among the individual pepper samples showing virus-like symptoms such as malformed fruits, ring spots, and necrotic lesions, the incidence of single TSWV infection was calculated to be 57% (Beşkeçili *et al*., 2021). In Konya province, TSWV was identified as the most prevalent virus in 160 tomato plants showing symptoms of mosaic, deformation, yellowing, blight, and stunted growth using the DAS-ELISA method, with a relative infection rate of 48.75% (Yeşil, 2022). Furthermore, with varying rates of infection, TSWV has been documented in tomato crops across different regions, including Amasya, Samsun, and Tokat (Şin, 2015); Mersin (Küçük and Kamberoglu, 2008); Isparta and Burdur (Çulal Kılıç *et al*., 2017); Çanakkale (Turhan and Korkmaz, 2006); Edirne (Yılmaz, 2014); Bursa, Bilecik, Sakarya, and Tekirdağ (Değirmenci and Uzunoğulları, 2007); Bursa and Yalova (Geyik, 2017); and Hatay (Sertkaya and Yılmaz, 2017).

Research investigating the phylogenetic relationships of the virus based on its nucleotide sequence is relatively scarce. Only two research on TSWV have been conducted in Denizli province, Türkiye, according to the current literature review. In one study, the presence of TSWV was detected in greenhouse tomatoes and certain weed species during the 2005 and 2006 growing seasons. ELISA tests revealed that 43 of 71 leaf samples exhibiting TSWV-like symptoms tested positive for TSWV. Additionally, this study identified seven weed species from nine different families as potential reservoirs for TSWV (Özdemir *et al*., 2009). In another study by Güneş *et al*. (2022), the prevalence and possible sources of TSWV were determined in tobacco fields in Denizli province. DAS-ELISA tests revealed that 31.2% of the sampled 179 tobacco plants and 10 out of 243 weeds were TSWV-positive.

Morca *et al*. (2022) investigated the prevalence and molecular characterization of TSWV with the RT-PCR test and reported that TSWV infection was 43.6% in tomato, pepper and *Chenopodium album* plants. Out of 148 pepper samples, 53 (35.81%) were found to be infected with TSWV using the DAS-ELISA method. Subsequent to obtaining the positive samples, they had further analysis via RT-PCR, utilizing specific primers that targeted the nucleocapsid protein gene, glycoprotein gene, and RNA-dependent RNA polymerase gene of the TSWV genome. The nucleotide similarity rates of the identified TSWV isolate's gene regions were found to be between 92-98%, indicating a similarity to other isolates in the GenBank database. A phylogenetic tree constructed based on the S RNA segment revealed that the TSWV-Antalya isolate was closely related to isolates from *Datura stramonium* and *Nicotiana sylvestris* from Samsun, Türkiye, peppers from New Zealand, *Chrysanthemum* sp. from South Korea, and peppers from Italy.

In Türkiye, a comprehensive and current study has provided insights into the genetic diversity and population structure of TSWV. The study focused on TSWV isolates from tomato, pepper, and *Chenopodium album* plants in various provinces, including Ankara, Eskişehir, Bartın, Zonguldak and Konya. The findings revealed that the N gene of TSWV experiences strong negative selection pressure across all analyzed populations. Additionally, the populations were found to undergo balancing selection through three neutrality tests. Genetic differentiation and gene flow analyses demonstrated that the populations exhibit distinct characteristics, with rare gene flow occurring between generations (Morca *et al*., 2022).

In our study, TSWV isolates clustered independently of their hosts and geographic origins. This could be attributed to various factors, including shared genetic characteristics, evolutionary history, a common origin, or transmission by the same vector. The genetic similarity of a plant virus is influenced by the processes of evolution. During evolution, random mutations and recombinations can occur in the genetic material of virus. A specific virus species can infect different plant species in diverse geographic regions, experiencing different selection pressures in those plants. These selection pressures can lead to distinct mutations and recombinations in the viruses, resulting in variations in their genetic material. The presence of similar genetic features among viruses of the same species in different geographic regions and plant species may be a consequence of the evolutionary process. Indeed, the clustering of viruses in distinct phylogenetic groups can provide valuable insights to scientists regarding the evolution and spread of these viruses.

Currently, the discovery of an effective antiviral reagent for total protection against TSWV infection remains a challenging task. However, ribavirin and ningnanmycin have been widely used as antiviral agents against several plant viruses such as TMV, TSWV, PVX, and PPV (Quecini *et al*., 2008; Li *et al*., 2017; Oana *et al*., 2009; An *et al*., 2019; Paunovic *et al*., 2007). Tunicamycin, an N-glycosylation inhibitor, has also been employed as an antiviral agent against TSWV (Kikkert *et al*., 2001).

Phytochemicals are natural compounds found in plants that play important roles in their development, growth, and defense mechanisms. Some phytochemicals have been shown to possess antiviral properties and may be valuable in preventing plant virus infections. *In silico* techniques, which involve computer-based strategies, are used to study biological systems. I*n silico* approaches can be employed to predict and evaluate the interactions between phytochemicals and viral components, allowing researchers to investigate the potential impact of phytochemicals against plant viruses. In this study, homology modeling was conducted for two coat proteins of TSWV isolates from pepper and tomato in the Denizli province of Türkiye. Additionally, commonly used antiviral agents for TSWV, including ribavirin, tunicamycin, and ningnanmycin, as well as phytochemicals such as catechin, curcumin, and quercetin, were subjected to virtual screening to identify the most effective compounds for inhibiting TSWV. The research findings revealed that among the antiviral agents tested against TSWV, epigallocatechin exhibited the highest docking score with the TSWV CP. Soundararajan *et al*. (2014) demonstrated that ribavirin and tunicamycin interact with the G_C and G_N glycoproteins of TSWV through protein-ligand docking analysis. They further reported that tunicamycin displayed a higher docking score than ribavirin for the TSWV G_N protein. Consistent with their findings, our docking results for TSWV CP also indicated that tunicamycin had a higher docking score than ribavirin. Jeyaraj *et al*. (2021) conducted molecular docking studies between various flavonoids, chemicals, and the CP protein of chilli leaf curl virus (ChiLCV). Their results revealed that flavonoids exhibited greater binding energies than chemicals, highlighting the potential of flavonoids as effective inhibitors.

Conclusions

The present study is the first phylogenetic and molecular report of TSWV isolates infecting tomato and pepper plants in the province of Denizli, Türkiye. In this study, TSWV was detected pepper and tomato plants. The infection rate was calculated as 29.48% in 23 out of 78 samples (10 pepper and 13 tomato samples). The complete coat protein gene of three isolates, comprising two peppers and one tomato, was recorded in GenBank, with a length of 777 nucleotides. Moreover, the relationship between these isolates and different isolates around the world has been studied. The phylogenetic dendrogram grouped the Turkish-Denizli isolates with the isolates from Hungary, Türkiye, and Serbia. Sequencing and genetic comparison studies showed that they were very similar to each other and to samples from all over the world.

In conclusion, a physicochemical evaluation of the coat protein (CP) of Denizli Tomato spotted wilt virus (TSWV) isolates was carried out, and it was shown that all of the isolates had very similar values for most physicochemical parameters except for EC and II. According to the II index, TSWV isolate CP proteins are relatively stable. The SOPMA secondary structure analysis revealed that the CP proteins have mostly alpha helics. The CP proteins' three-dimensional structures were modeled using Phyre2, Swiss-Modeler, and I-TASSER, all of which showed a significant degree of similarity to the c5ip1A template. The stereochemical quality of the models was verified using Ramachandran plot analysis. Docking analyses were carried out in order to evaluate the binding affinities of selected antiviral compounds and phytochemicals. According to our knowledge, this study is the first modeling and docking report on CP of TSWV. Tunicamycin (-7.6) exhibited the highest docking energy among the commercially available antiviral agents, followed by ningnanmycin and ribavirin, respectively. The phytochemical with the highest docking energy for the CP of TSWV was epigallocatechin (-8.6), followed by catechin, quercetin, curcumin, and chalcone, respectively. These results indicate that these chemicals may be useful as TSWV inhibitors. The utilization of *in silico* techniques presents a significant opportunity for investigating the interactions between phytochemicals and viral components, thereby facilitating the exploration of potential avenues for the prevention and control of plant viruses. In summary, this study enhances our comprehension of the physicochemical attributes and structural features of CP proteins of TSWV. These findings provide a framework for future research attempts and the development of effective approaches for dealing with TSWV and other plant viruses.

Authors' Contributions

AG and MU planned the study, collected the samples, provided sequencing, and wrote the manuscript. GA, MU and SD wrote and edited the manuscript. SD, GK, ZK performed laboratory studies and conducted phylogenetic and *in silico* analyses. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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