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ORIGINAL ARTICLE

Population genetic dynamics of southern tomato virus from Turkey

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Abstract

Southern tomato virus (STV) has been reported from many countries. However, the infection status and population structure of STV in Turkey are unknown. In this study, the presence of STV was investigated in eight provinces across three different geographical regions of Turkey, including the Mediterranean, Aegean, and Marmara regions during the 2019 year vegetation period. Samples from 127 symptomatic and 9 asymptomatic plants from tomato production areas in three different geographical regions of Turkey were tested for a range of tomato infecting viruses using conventional molecular assays. Over 54% of the tomato plants sampled were found to be infected with STV. Also, single, double, and triple infections caused by STV, CMV, a potyvirus, and 16SrXII-A '*Candidatus* Phytoplasma Solani' subgroup were detected. The genetic diversity of STV was investigated using the complete coat protein (CP) and RNA-dependent RNA polymerases (RdRp) gene regions of 15 randomly selected STV variants from different geographical regions of Turkey, and global variants accessions from GenBank. Variants from Turkey and other countries showed more than 99% homology with each other. According to the complete CP and RdRp gene regions, phylogenetic analyses revealed two lineages independent of geographical origin and host, and it was observed that all Turkish STV variants clustered in Clade I. Based on the complete CP and RdRP genes of STV, genetic parameters for Turkish and other international variants demonstrated high haplotype diversity and low nucleotide diversity. Negative (purifying) selection, a mechanism that constrains genetic variation, was determined on either CP and RdRp genes of STV. Moreover, based on our best knowledge this study is the first report of STV in Turkey.

Keywords Tomato · STV · Diversity · Phylogenetic · Selection pressure

Introduction

Tomato (*Solanum lycopersicum* L.), a member of the Solanaceae family, is one of the most cultivated vegetables worldwide. An average of 180 million tonnes of tomatoes are globally cultivated today, with production increasing year on year (FAO 2021). Tomato, which has a wide range of climatic tolerance, is grown in different production systems such as open fields, polytunnels, and glasshouses. Turkey, which has a broad climate zone, is among the important tomato producers with an annual yield of approximately 13 million tons (FAO 2021).

Viruses, fungi, bacteria, and phytoplasmas can infect tomato plants, causing a severe impact on production (Panno et al. 2021). Virus infections, in particular, can lead to lower product yield, low nutrient content, shorter shelf life, and poor fruit quality, making them unmarketable (Hanson et al. 2016). Over 130 viruses and virus-like pathogens have been reported from tomato plants (Hanssen et al. 2010). There are many established viruses of tomato such as tomato spotted

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wilt virus (TSWV), tomato yellow leaf curl virus (TYLCV), tomato infectious chlorosis virus (TICV), cucumber mosaic virus (CMV), pepino mosaic virus (PepMV), and tomato chlorosis virus (ToCV) (Roselló et al. 1996; Navas-Castillo et al. 2011; Duffus et al. 1996; Rendina et al. 2019; Van der Vlugt et al. 2011; Wintermantel and Wisler 2006). Recently, there have been reports of emerging viruses inducing severe constraints on tomato production including torradoviruses and tomato brown rugose fruit virus (ToBRFV) (Van der Vlugt et al. 2015; Oladokun et al. 2019).

The foliar symptoms of virus infections can range from asymptomatic to various symptoms and can be confused with damage caused by other biotic and abiotic factors. More specifically, infections in tomatoes by viruses such as ToBRFV, PepMV, TSWV, and torradoviruses have a negative impact on fruit quality, resulting in uneven ripening, fruit damage, and reduced size (Roselló et al. 1996; Roggero et al. 2001; Van der Vlugt et al. 2015; Oladokun et al. 2019). Management of virus infections is challenging due to a lack of antiviral products that prevent viral infections (Hanssen et al. 2010), therefore, control of viral infections is based on the genetic resistance of the host, cultural measures (agronomic and phytosanitary), biological control, and legislation (Roger and Jones 2006).

Southern tomato virus was described as a member of the genus *Amalgavirus* from the family *Amalgaviridae* (Sabanadzovic et al. 2009). STV was first reported in 2009 from plants with dwarfism, colour change in fruits, and reduction in fruit diameter in tomatoes grown in America and Mexico (Sabanadzovic et al. 2009). Although the potential pathogenic role of the viral agent in symptom development is indefinite, it has been shown to cause symptomatic or asymptomatic infections in tomatoes (Puchades et al. 2017; Oh et al. 2018; Elvira González et al. 2021). The virus has been known to be transmitted at a high rate through seed, but there has been no evidence of horizontal, mechanically, or grafting transmission (Sabanadzovic et al. 2009). Known natural hosts of the STV are tomato (Sabanadzovic et al. 2009), pepper (*Capsicum annuum*) (GenBank, LC487710), hand spice (*Solanum nigrum*) (Ma et al. 2020), and cape gooseberry (*Physalis peruviana* L.) (García et al. 2020). Following the discovery of the virus, its presence has been documented in Asia, Europe, and North America continents (CABI 2021). Given the many reports of asymptomatic infections and the presence of NCBI sequence accessions from countries where the virus has not yet been reported, it is likely that the virus is more widespread than currently reported.

Southern tomato virus (STV) has a double-strand RNA genome, about 3.5 kb in length, with two overlapping ORFs (Open Reading Frames) regions. A putative coat protein (CP) and the RNA-dependent RNA polymerase are encoded by the ORF1 and ORF2, respectively (Sabanadzovic et al. 2009; Wickner et al. 2012). RNA-dependent RNA polymerase (RdRp), a well-rounded enzyme of RNA viruses, is essential for carrying out replication and transcription of the viral genome (Venkataraman et al. 2018). RdRps have a high error rate during the copying process, due to a lack of a proofreading exonuclease activity (Elena and Sanjuan 2005). However, the RdRp is the most conserved gene among RNA viruses, allowing it to be used to track the evolutionary history of viruses (Krupovic and Bamford 2009). Coat proteins (CPs) of plant viruses possess multifunctional properties (Callaway et al. 2001) being implicated in every aspect of viral multiplication and dissemination, and thus they play a critical role in virus survival in both plant and invertebrate ecosystems (Chare and Holmes 2004).

Studies on the genetic diversity of STV have noted that all variants show very high sequence homology with each other (Elvira-González et al. 2020), although studies on the virus's genetic diversity are still quite limited. Therefore, this study presents an analysis of the genetic diversity of STV variants, including both recently detected Turkish variants and those around the world. Additionally, this study investigated the evolutionary mechanisms that shaped their population structure. First, conventional molecular techniques (Reverse Transcription Polymerase Chain Reaction, RT-PCR) were used to detect STV variants from Turkey and to reveal more about virus symptomatology and epidemiology by screening for infections with major viruses and phytoplasmas. Moreover, the study utilized nucleotide and haplotype diversity estimation, selection pressure analysis, neutrality testing, and phylogenetic analysis based on the CP and RdRp genes to better understand the molecular development and diversification of STV.

Materials and methods

Collection of tomato samples

The field surveys were conducted in eight provinces across three different geographical regions of Turkey, including the Mediterranean (Antalya), Aegean (İzmir and Manisa), and Marmara regions (Tekirdağ, Edirne, Çanakkale, Balıkesir, and Bursa), all of which have tomato production areas during the 2019 year vegetation period (Fig. 1).

Totally, 127 symptomatic and 9 asymptomatic (3 samples from each region) tomato samples were collected, 20 from the Mediterranean region, 42 from the Aegean region, and 74 from the Marmara region. Also, tomato cultivating areas were selected at random from regions of the highest tomato production, and leaf samples were taken from up to six symptomatic plants per field. The samples were transported to the laboratory in a cool box and stored dry in the refrigerator over silica gel until analyzed.

Fig. 1 Geographical regions and sample numbers where tomato samples were collected

TNA extraction and PCR amplification

Total nucleic acid (TNA) isolation was performed by applying the protocol suggested by Li et al. (2011). Synthesis of cDNA was performed for the detection of RNA viruses using the cDNA synthesis kit (Takara, Japan) following the manufacturer's protocol. Primers were designed to select STV and amplify the region corresponding to the entire RdRp and CP gene regions (Table 1).

Primer sets were designed using Primer3web version 4.1.0 software (Koressaar et al. 2018) with the Florida STV variant (KX949574) selected as a reference after all STV sequences from GenBank were aligned. Degenerate potyvirus, polerovirus, tobamovirus, torradovirus, and CMV-specific primers were also used with a commercial PCR kit (Takara, Japan) to detect other RNA viruses (Table 2). In addition, previously reported degenerate and universal primers were used in PCR assays for the determination of geminiviruses, and nested-PCR was applied using 16SrRNA gene-specific universal primer pairs (P1/P7 and F2n/R2) for the detection of phytoplasmas (Table 2). In the first step reaction, about 30–50 ng/ul DNA was used as a template, and then the first PCR product was diluted with 1:30 nuclease-free water and the second step reactions were carried out. To determine the phytoplasma group of fragments obtained from 16SrRNA regions using F2n/R2 universal primers, digestion profiles were determined by the *Taq*I restriction enzyme (Eurx, Estonia) with reference variants. All reactions were performed in a thermal cycler (BioRad, USA) and the resulting products were visualized on 1% agarose gel stained with ethidium bromide and checked on a UV imager (Major Science UVDI, ABD).

Table 1 Primer pairs used in reverse transcription -polymerase chain reaction

Code	Primer Sequence (5'-3')	Sense	Locus	Amplicon Size (bp)	Amplification Sites	Reference
STV_ CPF*	CTCGTCGTTGCTTCCGTT	Forward	98_115	1310	Complete CP Partial RdRp	This study
STV_ CPR*	ACCACCACCCCTGTACTT	Reverse	1390_1407			
STV_F1	TCACTCCTCTATCCCAAC	Forward	1237_1254	1129	Partial CP Partial RdRp	
STV_R1	GCTTAACCCTCATTTCTTC	Reverse	2347_2365			
STV_F2	TGGTGGAAAGGAGATTGA	Forward	2123–2140	1290	Partial RdRp	
STV_R2	ATTAAGAACTGCCGAGTG	Reverse	3395_3412			

*This primer pair was also used in southern tomato virus detection studies. These primer pairs were designed on Florida isolate of southern tomato virus (KX949574)

Table 2 Primers used in virus and phytoplasma detection studies

Virus Genus/Species Groups	Primer Sequence (5'-3')	Sense	Expected Amplicon Size	Reference
Potyvirus	GTITGYGTIGAYGAYTTYAAYAA TCIACIACIGTIGAIGGYTGNCC	Forward Reverse	350	Zheng et al. 2010
Polerovirus	GAYTGCTCYGGYTTYGACTGGAG GATYTTATAYTCATGGTAGGCCTTGAG	Forward Reverse	1100	Knierim et al. 2010
Tobamovirus	ATTTAAGTGGASGGAAAAVCACT GTYGTTGATGAGTTCRTGGA	Forward Reverse	800	Letschert et al. 2002
Geminivirus	ACNGGNAARACNATGTGGGC GGNAAR-ATHTGGATGGA	Forward Reverse	1300	Rojas et al. 1993
Torradovirus	TGGGATGARTGYAATGTKCT CCWGTCCACCAYTTGCAATT	Forward Reverse	515	Verbeek et al. 2012
Phytoplasmas universal	AAGAATTTGATCCTGGCTCAGGATT CGTCCTTCATCGGCTCTT	P1 P7	1.8 kb	Deng and Hiruki (1991)
	GAAACGACTGCTAAGACTGG TGACGGGCGGTGTGTACAAACCCCG	F2n R2	1.2 kb	Gundersen and Lee (1996)
CMV	ATGGACAAATCTGAATCAACC GATGTGGGAATGCGTTGGTGC	Forward Reverse	638	Karanfil and Korkmaz 2017

Molecular characterization and sequencing

A total of 15 STV variants, at least one sample from each province, were selected among the samples in which infection was detected. The entire protein-coding region of the selected variants was amplified using 2X Emerald PCR Master Mix (Takara, Japan) with overlapping PCR using the primer pairs in Table 1. Amplicons were directly sequenced bidirectionally and the obtained raw sequence data were combined in CLC Main Workbench V.20 program and uploaded to GenBank after BlastN analysis (Supplementary Table 1).

Phylogenetic and recombination analyses

Nucleotide sequences containing complete RdRp and CP genes of the virus were obtained from GenBank to use in phylogenetic analyses, and a data set covering Turkish variants and World variants was created (Supplementary Table 1). For this reason, the sequences of partial CP and RdRp genes were not used further in the analyses. The phylogenetic relationships of the STV with worldwide variants were determined by the Maximum-likelihood (ML) method using the parameters obtained as a result of the hierarchical likelihood ratio tests (hLRT) performed after ClustalW alignment in the CLC Main Workbench V.20 software (Qiagen, Canada). The genetic distance values of the variants were calculated by the maximum composite likelihood method, applying the bootstrap method with 1000 replications in the MEGAX program (Kumar et al. 2018). Also, outgroups included blueberry latent virus (BBLV) (MN416031), spinach amalgavirus 1 (SpAV1) (NC035070), and allium cepa amalgavirus 1(AcAV1) (NC036580) variants, which were used to construct phylogenetic trees from both the CP and RdRp gene regions. Sequence similarity ratios of STV variants were determined by sequencing ClustalW in the sequence demarcation tool V.1.2 program based on RdRp and CP genes at the nucleotide (nt) and amino acid (aa) (Muhire et al. 2014).

Recombination sites in the complete protein-coding genome and gene regions of STV isolates were assessed using RDP, GENECONV, BootScan, MaxChi, Chimaera, and SiScan recombination detection programs implemented in the RDP5 software (Martin et al. 2021). For the analysis, a *P-value* of less than 1.0×10^{-6} with at least three different methods were needed to consider true recombination (Ohshima et al. 2016). Recombinant-free variants were used for further analysis in this study.

Selection pressures analyses and tests of neutrality

Since there has been a limited number of STV variants from different geographical regions of the world, the variants were clustered into four groups Mediterranean "MED" (Turkey, Spain, Israel), European "EU" (Germany, Switzerland, Serbia, France, and UK), American "AME" (USA, Panama, Colombia, Dominican Republic, Mexico, Canada, and Brazil) and Asian "ASN" (Pakistan, China, Vietnam, Japan, Bangladesh, Thailand, and South Korea). In addition, a Réunion STV variant was not included in these analyzes, except for its phylogeny, since it is the only variant of the geography in which it is located. Thus, the analyses were carried out according to these four geographical groups and clades formed in the phylogenetic tree.

The selective pressure acting on CP and RdRp genes was assessed for all variants in four geographical groups and main phylogroups based on the relative rates of synonymous (dS) and nonsynonymous (dN) substitutions, ω = dN/dS [p(a)/p(s)], using DnaSP version 6.10.01 (Rozas et al. 2017) software. When the ω value is > 1, = 1, and < 1, the gene is under positive (diversifying), neutral, and negative (purifying) selection, respectively (Rozas et al. 2017). To determine the natural selection and population balance among the variations of nt sequences between phylogenetic groups and geographical populations Fu and Li's F* and D* (Fu and Li 1993) and Tajima's *D* (Tajima 1989) tests of neutrality were implemented using the DNASP software for the CP and RdRp genes of STV.

Genetic diversity and haplotype networks of STV

Genetic diversity analyses were performed in the DnaSP V6.12.03 software based on the CP and RdRp of STV gene regions. Haplotype diversity (H_d), haplotypes (H), and average pairwise nucleotide diversity (π) were calculated according to geographical groups and phylogenetic groups. The 846 nt was removed from the sequence alignment during the analysis due to ribosomal frameshifting in the RdRp protein gene region in the STV genome (Nibert et al. 2016). Haplotype networks were designed to represent the genetic variation and evolutionary relationships of aligned sequences for each of the STV Cp and RdRp genes at the geographic level. The Median Joining (MJ) algorithm (Bandelt et al. 1999) was used to create the haplotype networks, which were then visualized using PopART software (http://popart.otago.ac.nz) (Leigh and Bryant 2015).

K_S*, Z*, S_{nn}, F_{ST}, and the number of effective migrants (Nm) test statistics (Hudson et al. 1992; Hudson 2000) were calculated in DnaSP software to predict genetic differences, migration rates and gene flows among STV populations based on CP and RdRp gene regions.

If there is no genetic differentiation (null hypothesis), K_S* will be near to zero (Tsompana et al. 2005), and the lower the Z*, the less genetic differentiation there is among populations (Hudson et al. 1992). While the fixation index (Fst) value is 0, it indicates that there is no genetic differentiation between populations, while the value of 1 indicates that there is a complete differentiation between populations (Hudson 2000). More specifically, based on Wright's F-statistics (Wright 1965), Fst calculates the quantity of genetic variance that could be described by population dynamics, when Nm = [(1/Fst)-1]/4. Also, the value of Fst > 0.33 or Nm < 1 indicates low gene flow, whereas the value of Fst < 0.33 or Nm > 1 suggests high gene flow between populations.

Results

Survey results

During the field surveys, a total of 127 symptomatic and 9 asymptomatic tomato samples were taken from plants showing foliar symptoms typically caused by viruses and virus-like agents, such as mosaic, leaf deformation, leaf mottling, yellowing, and leaf curling (Fig. 2).

It was determined that 98 out of 127 (77.16%) symptomatic tomato plant samples were infected by at least one or more pathogens (Table 3). Moreover, no positive results were obtained against any pathogen from 9 asymptomatic plants. PCR tests indicated that none of the samples was infected with geminivirus, torradovirus, tobamovirus, and polerovirus. Furthermore, the infection combination of STV, CMV, potyvirus, and phytoplasma infections (single, double, and triple infections) were determined. Phytoplasma infections caused by the 16SrXII-A '*Candidatus* Phytoplasma solani' subgroup were revealed by the *Alu*I enzyme digest profile and sequence analysis (data not shown). Single infections caused by STV were detected in 15 out of 98 samples where a pathogen had been detected. Mixed infections of STV with one other pathogen were detected with CMV in 34 (26.77%) samples, with a potyvirus in 8 samples (6.30%), and with phytoplasmas in 6 samples (4.72%). In addition, triple infections caused by STV, CMV, and phytoplasma were detected in 6 plant samples (4.72%). From the pathogen-positive samples without STV, single infections were detected caused by CMV, potyvirus, and phytoplasmas in 17 samples (13.39%), 7 samples (5.51%), and 5 samples (3.94%), respectively. In total, of the 98 pathogen-positive samples, 69 samples (70.41%) were infected with STV (single, double, and triple infections), indicating a 54.33% STV infection rate from all samples tested.

Phylogenetic relationships

Phylogenetic analyses were performed using the nt sequences of the RdRp and CP genes to evaluate the evolutionary relationships between the STV variants. Two phylogenetic trees with similar topological features emerged as a result of these analyses (Fig. 3). A total of 68 CP and 52 RdRp sequences originating from variants from different geographies of the world and the Turkish variants from this study, were separated into two main lineages as Clade I and Clade II in the phylograms. The divergence points of these lineages were supported by high confidence values. A total of 5 variants, two from Switzerland, 2 from Germany and 1 from China, were clustered in the Clade

Fig. 2 Symptoms caused by viruses and virus-like agents observed in tomatoes **a** yellowing (STV + potyvirus), **b** and **c** mosaic, leaf deformation (CMV + STV) **d** leaf deformation (16SrXII-A subgroup phytoplasma + STV)

II lineage for both gene regions. In addition, 63 CP and 47 RdRp variants, including Turkish variants, clustered in Clade I (Fig. 3).

To confirm the phylogenetic analysis, the genetic distances of the two main lineages and geographic variants within themselves and between each other were calculated. The genetic distance between Clade I and Clade II was 0.0144 ± 0.0020 and 0.0116 ± 0.0031 for the RdRp and CP gene regions, respectively. For the CP gene region, the genetic distance within the Clade I population had a value of 0.0016 ± 0.0003, while for the RdRp gene region, this value was 0.0026 ± 0.0004. The genetic distances within the Clade II variants were 0.0033 ± 0.0007 and 0.0012 ± 0.0007 for the RdRp and CP gene regions, respectively.

Considering the RdRp gene region from a geographic perspective, European and Mediterranean populations had the furthest inter-regional genetic distance with a value of 0.0071 ± 0.0008, while Asian and American populations were genetically closest to each other with a value of 0.0025 ± 0.0004. For the CP gene region, populations from Europe and Asia were genetically farthest away with a value of 0.0056 ± 0.0013, while Mediterranean and American populations were genetically closest to each other with a value of 0.0011 ± 0.0003. The European variants had the greatest intra-regional distance for both gene regions (RdRp gene, 0.0076 ± 0.0011 and CP gene, 0.0063 ± 0.0017). This may be due to the influence of genetic variation between the four European variants in Clade II and the other variants from Europe in Clade I on the formation of lineages.

Population dynamics of STV

Viral RNA genomes can replicate rapidly with lacking proofreading features which cause their genomes to be subject to selective pressures, which can significantly affect their evolutionary history. Therefore, to better understand the evolutionary process of the CP and RdRp genes, genetic parameters were assessed using phylogenetic lineages (Clade I and II) variants and different geographic STV populations (Table 4). The nucleotide diversity (π) values for the CP gene sequences ranged from 0.00039 to 0.00602, and the haplotype diversity (H_d) values from 0.417 to 0.964 in two main lineages and geographic populations. The nt diversity (π) values for RdRp gene sequences ranged from 0.00142 to 0,00,748, and H_d values from 0.917 to 1.000 for clades (I and II) and geographic populations. According to the π and H_d genetic parameter values obtained by using the two gene region sequences of STV, high haplotype diversity values (>0.917) and low nt diversity values (<0.00748) were found within lineages and populations, especially for the RdRp gene. Furthermore, the selection pressure that shaped the evolution of the virus and had an influence on the codons constituting the gene was found to be $\omega = (dN/dS) < 1$ for both the CP and RdRp gene regions in four geographic regions and two lineages population variants (Table 4). The highest ω values were calculated from Clade I (0.224138) and Mediterranean populations (0.287634), whilst the lowest ω values were calculated from Clade II (0.214511) and American populations (0) for the CP gene of STV. The values for the RdRp gene region with the greatest ω ratios were

Table 3 Infection distributions and rates of plant samples

Region	No. of plant samples	STV single	CMV single	Potyvirus single	Phytoplasma	STV + CMV double infection	STV + Potyvirus double infection	STV + Phytoplasma double infection	STV + CMV + Phytoplasma triple infection	Total infection rate
Marmara	71	7 (9.86%)	7 (9.86%)	2 (2.82%)	5 (7.04%)	17 (23.94%)	8 (11.27%)	4 (5.63%)	6 (8.45%)	78.87%
Aegean	39	6 (15.38%)	7 (17.95%)	3 (7.69%)	0 (0.00%)	11 (28.20%)	0 (0.00%)	2 (5.13%)	0 (0.00%)	69.23%
Mediterranean	17	2 (11.76%)	3 (23.53%)	2 (11.76%)	0 (0.00%)	6 (35.29%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	82.35%
Total	127	15 (11.81%)	17 (13.39%)	7 (5.51%)	5 (3.94%)	34 (26.77%)	8 (6.30%)	6 (4.72%)	6 (4.72%)	76.37%

* This infection table is arranged according to the test results obtained from 127 symptomatic tomato plants. Negative results from 9 asymptomatic plants are not included in this table

found from Clade I (0.28275) and Mediterranean populations (0.34367), whereas the smallest ω ratios were found from Clade II (0.27843) and Europe populations (0.05784).

To predict evolutionary changes operating on the CP and RdRp genes of STV between phylogenetic clades and four geographical populations, neutrality tests were implemented using Tajima's *D*, Fu and Li's D* & F* statistics (Table 5).

The results of Tajima's *D* and Fu and Li's D* & F* tests for both Clade I and Asian populations were statistically significant with negative values for both gene regions. These results may indicate that the population has been subjected to recent expansion, genetic hitchhiking, or background selection. Also, a negative value with statistical significance was found according to the Tajima's *D* test for Mediterranean populations consisting of Turkish, Spanish, and Israeli variants of the CP gene except Fu and Li's D* & F* statics results. The values of Tajima's *D* and Fu and Li D* & F* tests were not significantly positive in the European populations. There were no statistically significant positive or negative values for the neutrality tests in other Clade II, America, and the Mediterranean populations (except Tajima's *D* test result) for both genes, indicating that these populations might have been undergoing a contraction or a neutral period.

Haplotype networks

The haplotype network structure has been constructed to analyze and visualize the CP and RdRp gene regions of STV at the geographic population level (Fig. 4). A total of 25 haplotypes were determined and more than one haplotype was revealed in all except sixteen samples from 66 CP gene variants. More specifically, Hap_7 showed the highest prevalence and was geographically found in the Mediterranean, Asian, European, and American variants. Also, some variants from Turkey belonged to Hap_7. In addition, a total of 35 haplotypes were found, and a distinct 28 haplotype was identified in all but seven haplotypes from 50 RdRp gene variants. Of these haplotypes, Hap_6 showed a high prevalence and included variants from Asia, America, and Europe. However, haplotypes from different geographical regions, especially according to the CP gene region, seemed to be very closely related to each other. In addition, two main lineages (Clade I and II) obtained for both gene regions by phylogenetic analyses were revealed in these network analyses (Fig. 4). According to these results, the haplotype network may not be complete as the number of gene sequences obtained from different geographical origins was limited and more variants are needed to support further analysis.

Genetic differentiation and migration

Independent statistical tests based on permutation with 1000 replicates, Ks*, Kst*, Z*, Snn, Fst, and Nm, were used to

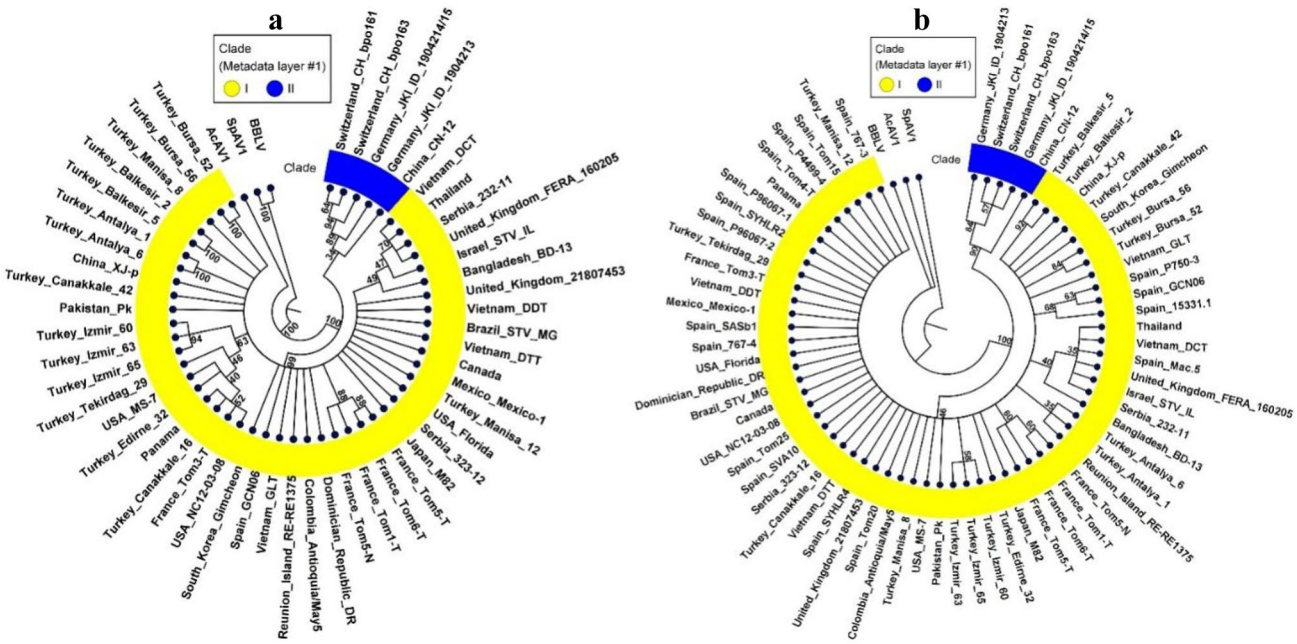

Fig. 3 Maximum-likelihood (ML) tree constructed from the RNA dependent RNA polymerase (RdRp) **a** and coat protein (CP) **b** coding region sequences of southern tomato virus (STV). Blueberry latent virus (BBLV) (MN416031), spinach amalygavirus 1 (SpAV1) (NC035070), and allium cepa amalgavirus 1 (AcAV1) (NC036580) variants were used as outgroups to construct phylogenetic trees from both the CP and RdRp gene regions

assess genetic differentiation and migration between/within two lineages and four populations originating from distinct locations (Table 6). For both the CP and RdRp gene regions of Clade I and Clade II phylogroups, the genetic differentiation values (Ks*, Kst*, and Z*) obtained as a result of the tests were statistically significant. The values showed genetic differentiation between the two phylogroups and were confirmed by a S_{nn} (=1,000) test result that was statistically significant. Furthermore, the absolute value of Fst between two lineages was greater than 0.33, and the absolute migration rate was less than 1. The significant statistical results between phylogroups obtained for both two gene regions were supported by the migration rate, indicating infrequent gene flow and high genetic variation among them. Additionally, for the CP and RdRp gene domain tests, small values of Ks*, Kst*, Z*, and S_{nn} were statistically significant among geographical populations consisting of the Mediterranean/Asian and Mediterranean/European variants. The results from these tests were strongly supported by an absolute value of Fst less than 0.33 and an absolute value of Nm greater than 1. Tests of genetic variation in other geographic populations revealed statistically significant or nonsignificant differences for each independent

Table 4 Results of genetic diversity parameters

Gene	Parameter	All (n = 66)	Clade I (n = 61)	Clade II (n = 5)	Mediterrenian (n = 33)	Europe (n = 13)	America (n = 9)	Asia (n = 11)
CP region	π	0.00299	0.00158	0.00124	0.00165	0.00602	0.00039	0.00388
	H	25	22	3	12	6	3	9
	H_d	0.814	0.790	0.700	0.759	0.846	0.417	0.964
	dN/dS	0.11247	0.224138	0.214511	0.287634	0.048264	0	0.16877
Gene	**Parameter**	**All (n = 50)**	**Clade I (n = 45)**	**Clade II (n = 5)**	**Mediterrenian (n = 17)**	**Europe (n = 13)**	**America (n = 9)**	**Asia (n = 11)**
RdRp region	π	0.00470	0,00260	0,00332	0,00396	0,00748	0,00142	0,00325
	H	35	30	5	12	10	7	9
	H_d	0.977	0,972	1,000	0,956	0,923	0,917	0,964
	dN/dS	0,13914	0,28275	0,27843	0,34367	0,05784	0,27076	0,18418

Table 5 Neutrality test results of geographic populations and two main lineages

Gene	Neutrality tests	All (n = 66)	Clade I (n = 61)	Clade II (n = 5)	Mediterrenian (n = 33)	Europe (n = 13)	America (n = 9)	Asia (n = 11)
CP	Fu and Li D*	-2.19526 ns	-3,66,862**	-0.17475 ns	-0.50142 ns	1.24595 ns	-1.50507 ns	-2.22089*
	Fu and Li F*	-2.59267 *	-3,84,988**	-0.17531 ns	-1.14051 ns	1.45629 ns	-1.62607 ns	-2.42096*
	Tajima's D	-2.12368 *	-2.47205**	-0.17475 ns	-1.85516 *	1.35639 ns	-1.36240 ns	-1.88803*
Gene	**Neutrality tests**	**All (n = 50)**	**Clade I (n = 45)**	**Clade II (n = 5)**	**Mediterrenian (n = 17)**	**Europe (n = 13)**	**America (n = 9)**	**Asia (n = 11)**
RdRp	Fu and Li D*	-2,09686 ns	-3,66862**	-0,86832 ns	-0,784495 ns	0,86590 ns	-0,73727 ns	-2,47757 **
	Fu and Li F*	-2.39347 ns	-3,84988**	-0,93355 ns	-1,06957 ns	1,04819 ns	-0,69467 ns	-2,69435 **
	Tajima's D	-1,84282*	-2.47205**	-0,86832 ns	-1,26358 ns	1,05074 ns	-0,25475 ns	-2,07232**

*Statistical significance: $P < 0.05$; **Statistical significance:, $P < 0.01$, $0.10 > P > 0.05$, $P < 0.02$, $P > 0.10$

test (Ks*, Kst*, Z*, and S_{nn}), which did not fully support each other. The results, however, all had low absolute values, indicating that there were no significant genetic differences between these populations based on the other absolute values of Fst (< 0.33) and Nm (> 1) (Table 6).

Discussion

Virus-virus interactions may influence viral properties and end up leaving a genetic signature in the population, contributing to viral disease prevalence and the emergence of novel variants (Alcaide et al. 2020). Thus, these interactions in plants may be critical for understanding viral pathogenesis and evolution, as well as developing effective and consistent control strategies (Syller 2012). Mixed infections by different unrelated viruses in a host can lead to the emergence of new diseases or lead to an increase in the virulence of co-infecting agents (Murphy and Bowen 2006; Syller 2012). The viruses involved in a co-infection can interact in a variety of ways with each other, ranging from synergism to neutralism to antagonism (Moreno and Lopez-Moya 2020). In a previous study, it was demonstrated that in co-infections caused by ToCV and TYLCV in tomatoes, more severe

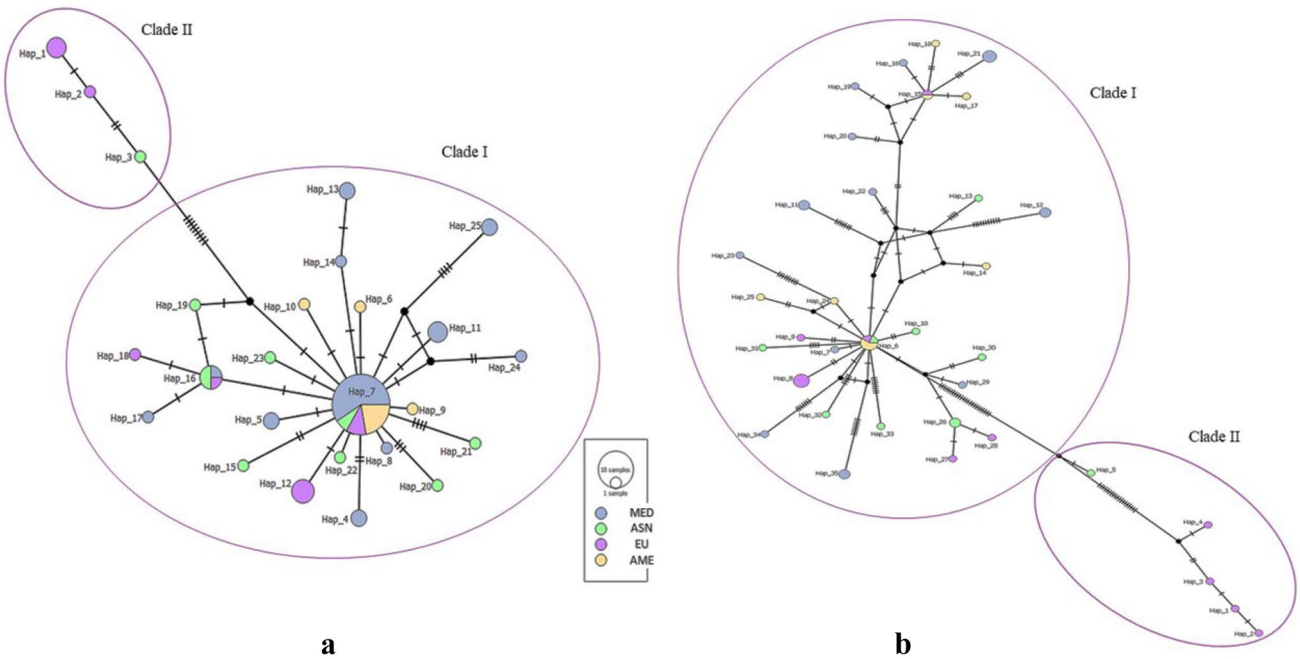

Fig. 4 Network analysis of STV haplotypes. A haplotype network was visualized using PopART software with the Median Joining algorithm. The colour of each node represents different geographical populations and allele types. MED: Mediterranean populations, ASN: Asian populations, EU: European populations, AME: American populations **a** Indicates haplotypes of the CP gene **b** Indicates haplotypes of the RdRP gene

Table 6 The values of genetic differences and gene flow analyze between populations and phylogroups based on the CP and RdRp gene regions of STV

Gene region	Statistical test	EU (n = 13) vs MED (n = 33)	MED (n = 33) vs ASN (n = 11)	MED (n = 33) vs AME (n = 9)	ASN (n = 11) vs EU (n = 13)	ASN (n = 11) vs AME (n = 9)	EU (n = 13) vs AME (n = 9)	Clade I (n = 61) vs CladeII (n = 5)
CP region	*Ks**	1.05358	1.00482	0,76,442	1.52740	0.95910	1.07797	0.85487
	(P value)	(0,0010 **)	(0,0100*)	(0,6000 ns)	(0,1190 ns)	(0.0130*)	(0.0220*)	(0,0000***)
	*Kst**	0,08,613	0,03,229	-0,00,713	0,02,632	0,05,663	0,10,513	0,23,167
	(P value)	(0,0010 **)	(0,0100*)	(0,6000 ns)	(0,1190 ns)	(0.0130*)	(0.0220*)	(0,0000***)
	*Z**	5.92536	5.87050	5.85891	4,59,885	4.20517	4.39879	6.58967
	(P-value)	(0.0010**)	(0,0160*)	(0,3510 *)	(0,0640 ns)	(0.0070**)	(0.0070**)	(0,0000***)
	S_{nn}	0.80544	0,71,626	0,68,910	0.58333	0.57333	0.73168	**1.00000**
	(P value)	(0,0000* **)	(0,0280*)	(0,2000 ns)	(0,1050 ns)	(0.0520 ns)	(0.0020**)	**(0,0000***)**
	Fst	0,19,116	0,02,239	0,02,701	0,07,037	0.02915	0.22445	0,87,765
	Nm	1.06	10.91	9.00	3.30	8.33	0.86	0,03
Gene region	**Statistical test**	**EU (n = 13) vs MED (n = 17)**	**MED (n = 17) vs ASN (n = 11)**	**MED (n = 17) vs AME (n = 9)**	**ASN (n = 11) vs EU (n = 13)**	**ASN (n = 11) vs AME (n = 9)**	**EU (n = 13) vs AME (n = 9)**	**Clade I (n = 45) vs Clade II (n = 5)**
RdRp Region	*Ks**	2,49,069	2,33,640	2,16,863	2,34,608	1,86,710	2,14,211	2,05,787
	(P value)	(0,0000***)	(0,0030**)	(0,0490*)	(0,1010 ns)	(0.1160 ns)	(0,0390*)	(0,0000***)
	*Kst**	0,05,390	0,03,141	0,02,270	0,02,958	0,01,676	0,05,243	0,13,659
	(P value)	(0,0000***)	(0,0030**)	(0,0490*)	(0,1010 ns)	(0.1160 ns)	(0,0390*)	(0,0000***)
	*Z**	4.92987	4.82363	4,71,021	4,57,738	4.23793	4.37579	5,90,137
	(P value)	(0.0000***)	(0,0020**)	(0,0290*)	(0,080 ns)	(0.1150 ns)	(0.0530 ns)	(0,0000***)
	S_{nn}	0.66667	0,81,429	0,67,308	0.65833	0.57190	0.70682	**1.00000**
	(P value)	(0,0580 ns)	(0,0040**)	(0,0730 ns)	(0,0470*)	(0.1470 ns)	(0.0290*)	**(0,0000***)**
	Fst	0,18,204	0,07,421	0,01,604	0,10,567	0.05618	0.21034	0,79,096
	Nm	1.12	3,12	15,34	2,12	4,20	0.94	0,07

PM test; Probability obtained by the permutation test with 1000 replicates ns, not significant; Statistical significance

* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$

symptoms occur than in plants with single infections, due to synergistic interactions between the viral agents (Li et al. 2021). On the other hand, mixed infections caused by tomato torrado virus (ToTV) and PepMV in tomato plants were thought to be associated with an antagonistic interaction due to a significant decrease in the titre of PepMV and a slight increase in the titre of ToTV during infection time (Moreno and Lopez Moya 2020; Gomez et al. 2009, 2010). Furthermore, in a study by Elvira-Gonzalez et al. (2021), a strong decrease in CMV titre was observed in double-infections of tomatoes with CMV and PepMV, while this antagonism was not observed in triple-infections of CMV and PepMV with STV. Thus, STV may be capable of eliminating antagonistic interactions, also called cross-protection, superinfection exclusion, or homologous interference.

Several viruses, on the other hand, are reported to be neutral or even beneficial to their host plants, resulting in mutualistic interactions such as increasing the resistance of plants to abiotic stress (González et al. 2021). TYLCV, which is generally known for its destructive effects on tomato plants, has been shown to reduce drought-induced activation of stress response proteins and metabolites under drought conditions to avoid an acute response that could possibly be fatal to the host; therefore, this indicated a striking mutualistic interaction between the host plant and its viral pathogen (Mishra et al. 2022). More specifically, Fukuhara et al. (2020) reported that seeds of the M82 tomato cultivar infected with the STV agent germinate at a higher rate, and infected plants produce more fruit than healthy plants as a result of the mutualistic interaction between virus and host.

STV infection is detected extensively in both symptomatic and asymptomatic plants (Elvira González et al. 2021). While some studies have shown that STV can have devastating results in co-infections (Elvira González et al. 2021), some studies have reported that the virus positively affects some plant growth parameters in a mutualistic relationship with the host plant (Fukuhara et al. 2020). In this situation, the epidemiology and symptomatology of the virus show great variability, especially when it is found as a mixed infection with different climatic conditions, agricultural practices, and other plant virus diseases. Therefore, further work is needed to determine the causal relationship between STV and disease (Fox 2020). Here, in this study, we aimed to reveal the distribution of STV in important tomato-growing areas of Turkey, its relationship with other

pathogens, and compare the genetic characteristics of Turkish variants with global variants.

Using conventional molecular techniques, we determined that in this study, 98 out of 127 (77.16%) symptomatic tomato plants exhibiting foliar symptoms such as mosaic, leaf deformation, leaf mottling, yellowing, and leaf curling were infected by at least one pathogen (Table 3). Double- infections caused by STV + CMV (26.77%) were commonly detected from symptomatic plants. In these cases, it is possible that STV may have contributed significantly to the onset of symptoms by increasing the titre of CMV as observed by Elvira-Gonzalez et al. (2021). In almost 5% of cases phytoplasma (16SrXII-A "stolbur") was also present with the STV-CMV co-infection. In these cases, viral symptoms rather than a specific "big bud" symptom were observed in co-infected plants. Infections with single stolbur phytoplasma and STV plus stolbur phytoplasma (16SrXII-A) were also found, particularly in plants with severe yellowing and stunting. This result is the first report of co-infection by STV with stolbur phytoplasma in tomatoes.

Viruses such as potato virus Y (PVY) and TYLCV have been reported to co-infect tomatoes with 16S ribosomal (XII-stolbur/ I-aster yellows/ V-elm yellows) phytoplasma strains previously (Gungoosingh-Bunwaree et al. 2013). Even though the symptoms caused by the agent as a single infection are unknown, symptomatic plants have been detected to have an STV single infection (Gaafar et al. 2019; Harju et al. 2021). In our study, STV (11.81%), CMV (13.39%), and potyvirus (5.51%) agents were identified as single infections in plants exhibiting symptoms. STV, on the other hand, has been shown to cause co-infections with the agents TSWV, TYLCV or PVY viruses (Verbeek et al. 2015; Iacono et al. 2015), and to cause symptoms in early infections through synergistic interaction with CMV or PepMV (Elvira-Gonzalez et al. 2021). All of these findings clearly indicated that the persistent STV, in combination with other phytopathogens or viruses, plays an important role in the development of severe symptoms in tomatoes. Moreover, in our study, no infection was detected in asymptomatic plants.

A total of 66 CP and 35 RdRp STV variants, variants from Turkey, had no recombination, as previous studies announced (Galipienso et al. 2021; Elvira-Gonzales et al. 2020). Two distinct lineages (Clade I and Clade II) emerged in cladograms as a result of molecular evolutionary analyses using both the complete CP and RdRp gene regions of STV, and the main nodes from which these lineages diverged were supported by high bootstrap values in our study. A similar tree topology with two major clades (Group A and B) was revealed using 28 STV putative CP (p42) gene sequence variants in another study (Elvira-Gonzales et al. 2021), and Turkish variants were clustered with Clade I or Group A variants. Furthermore, the genetic distance between phylogenetic groups for the putative CP (p42) gene was calculated as 0.0112 ± 0.0028 (Elvira-Gonzales et al. 2020), and the CP and RdRP gene regions were measured as 0.0144 ± 0.0020 and 0.0116 ± 0.0031, respectively, in our study. These findings were in line with those of the other study, and phylogenetic analysis confirmed that the virus evolved into two distinct lineages. Considering the topological similarities of these trees, which were revealed from the RdRp gene region covering a large part of the STV genome and the CP gene region overlapping with this gene, this suggests that evolutionary processes may occur simultaneously and in the same direction throughout the genome of the virus.

More specifically, in strongly structured populations in the same location, a sequence is expected to have its nearest neighbors, and the S_{nn} value approaches 1 as the populations in two different locations differ from each other (Hudson 2000). S_{nn} equal 1 value was found to be significant between two phylogenetic lineages of both gene regions. In addition, at the geographic level, a S_{nn} value closer to 1 was calculated for the RdRp gene only from the Mediterranean and Asian populations. Similarly, the logarithmic values of F_{st} ($\gtrsim 0.33$) and Nm ($\lesssim 1$) obtained from this study strongly supported genetic differentiation between phylogroups that contained both gene regions. In our study, obtained values of S_{nn} ($p < 0.05$), gene flow, and genetic differences confirm the results inferred from the phylogenetic tree reflecting the co-evolution of CP and RdRp genes. Consequently, the two major clades revealed in both gene regions did not reflect the geographic distribution or host specificity of the populations (Fig. 3), as previously documented by Elvira-Gonzales et al. (2020).

Predictions of selection pressures that play an active role in the biological evolution of STV were revealed based on both CP and RdRp gene regions. Herein, the dN/dS (ω) ratios for the CP and RdRp genes were 0.16877 and 0.18418 for all variants, respectively, while the values for both gene regions were determined as 0.09529 and 0.18896 in another study by Galipienso et al. (2021). These ω values indicated that both genes of STV populations are under purifying (negative) or stabilizing selection evolutionary constraints. To put it another way, both the CP and RdRp genes could have played a key role in the adaptive evolution of STV. Furthermore, the presence of overlapping reading frames in the case of viruses generally restricts synonymous replacements since substitutions that are synonymous in one reading frame may not be synonymous in another and this leads to negative selection (Hughes and Hughes 2005).

The genetic structure of viral populations is formed by gene flow (migration) between different geographical areas, plants, or different parts of the identical plant, and high migration favours genetic uniformity between populations, reducing global genetic diversity (Rubio et al. 2013; Moya et al. 2004). Ks*, Kst*, Z*, and S_{nn} statistical values were found to be highly correlated with each other and to have low values at the

level of geographic populations in our study (Table 6). In particular, these values obtained from the Fixation index (> 0.33) and migration rate (< 1) indicate that there is a high gene flow among the geographical populations with the exception of phylogroups and that they have low genetic variations. Furthermore, for both two gene regions, the absolute value of Nm was less than one, and the F_{st} value was less than 0.33 in European and American populations. This finding could be linked to the great genetic differentiation of German and Swiss variants compared with those of other European countries. Alternatively, more STV variants genetic information is required to obtain clearer analysis results. Genetic demographic analyses were used at the geographical population level to support these analysis results in both the CP and RdRp gene regions, and STV variants showed low nucleotide diversity, as previously reported by Galipienso et al. (2021). Also, haplotype diversity was high in these populations, and these results were mapped by haplotype network analyses. This situation may indicate a large number of haplotypes that are genetically very close to each other, and then indicate that the population has recently undergone an expansion phase. Thus, low nt diversity values suggest very minor variations among haplotypes, despite the large haplotype diversity. It has been reported in previous studies that STV is transmitted and spread by seed vertically (Sabanadzovic et al. 2009). The minor variations between haplotypes, very low genetic differences between variants, and intense gene flow between geographic populations, could support seed-borne transmission for the geographic distribution of STV (Table 6). Similar analyses also demonstrated that the entry and spread of grapevine fanleaf virus (GFLV) into vineyard areas were via the infected plant propagation material (Sokhandan-Bashir and Melcher 2012).

Neutrality tests (Tajima's *D,* Fu and Li F* & D*) applied to determine the growth of populations based on both CP and RdRp gene regions and to test the selective neutrality of nt variability of their gene regions were negative for Asia and Clade I populations including Turkish STV variants (Table 5). Neutrality test results for the CP (p42) gene region, on the other hand, were reported as negative and statistically insignificant in a previous study by Elvira-Gonzales et al. (2020). These contrasting results may be related to the number of samples in the population. Consequently, the neutrality test values reported here would have indicated that they arose from an overabundance of intermediate frequency alleles, and they could have also arisen from population bottlenecks, balancing selection, or structures.

Conclusions

Despite their prevalence, persistent plant viruses have received less attention in plant virology than acute or chronic viruses (Roossinck 2010). Therefore, more genetic information is needed to understand the life cycles, host adaptations, transport routes, and many other biological characteristics of persistent viruses. In this study, we evaluated the presence of STV, a persistent virus, in Turkey and its infections caused by other viruses/phytoplasmas. Based on the CP and RdRp gene regions of STV variants obtained from the Marmara, Aegean, and Mediterranean regions of Turkey, we attempted to understand the genetic diversity and population structure of the agent with bioinformatics analyses together with other variants from the world. Furthermore, molecular evolutionary findings did not reflect the geographic specificity of the virus. We also know that, at present, it is limited to infecting only Solanaceae members such as tomatoes, pepper, hand spice, and cape gooseberry. The transmission and spread of seeds to other geographies have been demonstrated using the parameters of gene flow and genetic diversity. As a result, necessary precautions regarding the seed trade must be taken to reduce the spread of the agent. From another perspective, could this virus evolve into an acute or chronic virus in the future by being influenced by evolutionary forces and other viruses with which it interacts synergistically? Moreover, this study is the first report of STV in Turkey.

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Author contribution All authors contributed to the designing and writing of the manuscript. Ali Karanfil collected and tested samples and obtained nearly full-genome sequences of STV variants. Filiz Randa-Zelyüt performed bioinformatic analyses and deeply interpreted them. Adrian Fox supervised the conceptualization and writing of the manuscript. All authors have read and approved the final manuscript.

Data Availability The datasets generated for this study areavailable on request to the first and corresponding author.

Declarations

Ethics statement This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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