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Research Article
(Araştırma Makalesi)

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Molecular characterization of tomato spotted wilt virus (TSWV) and cucumber mosaic virus (CMV) affecting tomato and pepper crops in Izmir Province

İzmir ilinde domates ve biber bitkilerini etkileyen tomato spotted wilt virus (TSWV) ve cucumber mosaic virus (CMV) etmenlerinin moleküler karakterizasyonu

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ABSTRACT

Objective: The objective of this study was to investigate tomato spotted wilt virus (TSWV) and cucumber mosaic virus (CMV) infections in tomato and pepper plants showing virus-induced symptoms in vegetable growing districts of İzmir, Turkey.

Material and Methods: Surveys were carried out in tomato and pepper plantations in 2019 and 2021, and the incidences of these viruses in the collected leaf samples were determined by RT-PCR. Nucleotide identities and phylogenetic relationships of the TSWV and CMV isolates with other isolates retrieved from the GenBank database were determined.

Results: The results of this study showed that tomato plants were infected at the same rate (21.50%) with TSWV and CMV. Out of the tested pepper samples, 64.15% were infected with TSWV and 25.47% with CMV. The results showed that, the identity rate of nucleoprotein region of TSWV isolates from tomato was 99-96% at nucleotide level while the isolates from pepper showed 100-95% identity. On the other hand, the capsid protein gene region of the tomato isolate of CMV had nucleotide identity rate of 98-95% with other isolates in GenBank, while that of its pepper isolates had 100-98% identity. Also, CMV isolates of this study showed close phylogenetic relationship with the CMV isolates of subgroup IB.

Conclusion: This study revealed the prevalence of TSWV and CMV in symptomatic tomato and pepper samples in İzmir province and some molecular properties of them.

Keywords: Pepper, RT-PCR, solanaceous crops, tomato, virus

ÖZ

Amaç: Bu çalışmanın amacı, İzmir'in sebze yetiştirilen önemli ilçelerinde virüs kaynaklı semptomlar gösteren domates ve biber bitkilerinde tomato spotted wilt virus (TSWV) ve cucumber mosaic virus (CMV) enfeksiyonlarının araştırılmasıdır.

Materyal ve Yöntem: Domates ve biber üretim alanlarında 2019 ve 2021 yıllarında sürveyler yapılmış ve toplanan yaprak örneklerinde bu virüslerin enfeksiyon oranları RT-PCR ile belirlenmiştir. TSWV ve CMV izolatlarının GenBank veri tabanından elde edilen diğer izolatlarla benzerlik oranları ve filogenetik ilişkileri belirlenmiştir.

Araştırma Bulguları: Çalışma sonucunda domates bitkilerinin aynı oranda (%21,50) TSWV ve CMV ile enfekteli olduğu belirlenmiştir. Test edilen biber örneklerinin %64,15'inin TSWV ve %25,47'sinin CMV ile enfekteli olduğu tespit edilmiştir. Sonuçlar, TSWV domates izolatlarının nükleokapsid protein gen bölgesinin nükleotid benzerlik oranının %99-96 olduğunu, TSWV biber izolatının ise %100-95 benzerlik gösterdiğini ortaya koymuştur. CMV-domates izolatının kapsid protein gen bölgesi GenBank'taki diğer izolatlarla %98-95 arasında, CMV-biber izolatınınki ise %100-98 arasında nükleotid benzerliğine sahip olmuştur. Ayrıca, CMV izolatları, alt grup IB CMV izolatları ile yakın filogenetik ilişki göstermiştir.

Sonuç: Bu çalışma, İzmir ilinde simptomlu domates ve biber örneklerinde TSWV ve CMV'nin bulaşıklık oranını ve moleküler özelliklerini ortaya koymuştur.

Anahtar sözcükler: Biber, RT-PCR, patlıcangil, domates, virüs

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum* L.) are the most cultivated Solanaceous crops globally. However, the production is affected by different viruses, including the species *Tomato spotted wilt virus* (TSWV), member of the genus *Orthotospovirus* in the family *Bunyaviridae*, and the species *Cucumber mosaic virus* (CMV), member of the genus *Cucumovirus* belonging to the family *Bromoviridae*. They may cause considerable quality and yield losses. TSWV is one of the 10 most destructive plant viruses worldwide (Scholthof et al., 2011), and CMV has been determined as the most common type of Cucumoviruses worldwide (Loebenstein & Lecoq, 2012). These infectious agents have tripartite RNA genome. TSWV genome consists of Large (8.9 kb), Medium (4.8 kb) and Small (2.9 kb) negative or ambisense RNAs designated as their relative sizes (Adkins 2000). RNA-dependent RNA polymerase (RdRp) is encoded by segment L (Chapman et al., 2003), cell-to-cell movement protein (NSm) (Li et al., 2009) and viral glycoproteins (Gn-Gc) (Sin et al., 2005) are encoded by segment M, while RNA-silencing suppressor (NSs) (Takeda et al., 2002) and nucleocapsid protein (N) (Li et al., 2015) are encoded by segment S. CMV has positive-sense single stranded RNAs attributed as RNA1 (3.3 kb), RNA2 (3.1 kb) and RNA3 (2.2 kb) (Roossinck, 2002). RNA-dependent RNA polymerase (RdRp) is encoded by segment RNA1 (Gal-On et al., 2000), RNA-silencing suppressor (2b) is encoded by segment RNA2 (Goto et al., 2007) whereas cell-to-cell movement protein (3a) and coat protein (3b) are encoded by segment RNA3 (Canto et al., 1997). The coat protein is translated from subgenomic RNA4 (1 kb) that is produced from full-length negative strand of RNA3, and some CMV strains contain satellite RNAs (RNA5) (~335 nucleotides) (Thompson et al., 2008).

The widely distribution of TSWV and CMV is the result of wide range of host-plants comprising weed species (Parrella et al., 2003) and highly polyphagous nature of their vectors. Multiple species of thrips (Thysanoptera: Thripidae), particularly *Frankliniella occidentalis,* transmit TSWV in a circulative and propagative manner (Nagata et al., 2002). CMV is transmitted by aphid species in a non-persistent manner, particularly by *Aphis gossypii* and *Myzus persicae,* which occur worldwide (Pinto et al., 2008).

It is noteworthy that TSWV and CMV are prevalent throughout the Mediterranean Basin (Roggero et al., 2002; Yardımcı & Eryiğit, 2006; Debreczeni et al., 2015) and recently in Asia (Jiang et al., 2017; Vinodhini et al., 2021), Africa (Karavina et al., 2016; Waweru et al., 2020) and North America (Lin et al., 2003; Batuman et al., 2017). Furthermore, the rapid increase of new variants arise makes difficult to control the viruses (Roggero et al., 2002). Breeding resistant cultivars through the use of resistant genes has proven to be the most efficient control approach for viral infections. The *Sw*-5 and *Tsw* genes have been found for tomato and pepper, respectively to confer resistance against TSWV. However, existing genetic resistance rapidly have overcome by the resistance-breaking isolates which are increasingly becoming as a serious, worldwide agronomic concern. The resistance gene can be overcome by an amino acid substitution in the TSWV movement protein, NSm for tomato cultivars, and RNA-silencing suppressor, NSs for pepper cultivars, according to a comparison of nucleotide and amino acid sequences (Batuman et al., 2017; Jiang et al., 2017). CMV isolates are classified into three subgroups based on serological characteristics, nucleic acid hybridization, and phylogenetic analyses: IA, IB, and II (Roossinck et al., 2002).

Turkey is one of the leading tomato (almost 14 million tons) and pepper (almost 3 million tons) producing country. (FAO, 2020). Until now, negative effects of virus diseases on tomato and pepper growing areas were reported in different parts of Turkey. The DAS-ELISA method is widely used in the studies conducted in tomato and pepper production areas (Arli-Sokmen et al., 2005; Arli-Sokmen & Sevik, 2006; Yardımcı & Eryiğit, 2006). During 2019-2020, leaf samples were collected, and RT-PCR tests confirmed TSWV infection in tomato and pepper from various provinces from Turkey except for İzmir (Beşkeçili et al., 2021; Morca et al., 2022). Currently, İzmir province has an important position in terms of tomato and pepper production and exportation (TUİK, 2022). CMV and TSWV are the viral agents may cause similar symptoms such as ring spots and malformation in those crops, especially in pepper. The presence of TSWV in the

tomato and pepper production areas of İzmir has yet to be determined. Previous studies have reported the prevalence of CMV determined using serological techniques like ELISA on pepper, but that of TSWV is still lacking (Gümüş, 1998). Although more sensitive techniques as well as serological methods have been used to identify these viruses in some studies in the region, they have not involved the attempts to characterize them molecularly.

The objective of the present study was to detect TSWV and CMV in symptomatic samples from commercial tomato and pepper cultivars grown in İzmir province in 2019 and 2021, and molecularly characterize them according to their partial capsid protein gene regions. Thus, the updated information may support development of effective strategies for the long-term control of these viruses.

MATERIALS and METHODS

Virus isolates

Field surveys were conducted in the districts of İzmir province in tomato and pepper cultivation seasons. Randomly selected tomato and pepper fields and greenhouses were surveyed. Ödemiş, Bayındır, Torbalı, and Tire were the four major production districts surveyed in 2019. The same crops were surveyed in the districts of Ödemiş, Bayındır, Torbalı, Menderes, and Tire in 2021. Virus-like symptoms observed on most plants included mosaic, ring spots, chlorotic and necrotic spots, leaf curl and necrosis. Samples were taken and brought to the laboratory in the cold chain. Samples were kept at -20°C for further analysis.

Molecular identification using reverse transcription-polymerase chain reaction (RT-PCR)

In this study, the presence of TSWV and CMV in the samples was determined by molecular methods. Total RNA was extracted from all samples using silica-based RNA extraction method as described by Foissac et al. (2001). A EzDrop 1000 Spectrophotometer was used to assess the quality and quantity of the extracted RNA. The OneScript® Plus cDNA Synthesis Kit (Applied Biosystems, USA) was used to carry out the reverse transcription (RT) step for producing complementary deoxyribonucleic acid (cDNA) from template RNA. cDNA synthesis was done as follows: a mixture of 5 µL plant total RNA, 1 µL of 10 µM random primers, 1 µL 10 mM dNTP Mix and 7,5 µL Nuclease-free H_2O was incubated at 65°C for 5 min and chilled on ice. Subsequently, recommended volumes of 5X Reaction Buffer for RT, OneScript Plus RTase (200 U/µL) and RNaseOFF Ribonuclease Inhibitor (40 U/µL) were added in a total reaction mixture of 20 µL. The reaction mixture was heated for 10 min at 25°C, and then for 50 min at 50°C before being incubated for 5 min at 85°C. cDNA was stored at -20°C until required. After the RT step, PCR reactions were set up in a total volume of 50 µL containing of PCR Master Mix II (Thermo Scientific, USA), 4 µL cDNA, 2 µL 10 µM Forward primer, 2 µL 10 µM Reverse primer and Nuclease-free H_2O as recommended by the manufacturer. In an automated thermal-cycler (Gene Amp PCR 9700 systems, Applied Biosystems, USA), PCR amplification was performed. For the 777 nt fragment of the nucleocapsid protein gene of TSWV, gene-specific primer pairs TSWV_F (ATGTCTAAGGTTAAGCTCAC) and TSWV_R (TTAAGCAAGTTCTGCGAGTT) as previously reported by Nour et al. (2013) were used. Amplification conditions included denaturation step for 4 min at 94°C and 40 cycles of 1 min at 94°C, 2 min at 52°C, 2 min at 72°C followed by a final extension step at 72°C for 10 min. CMV_F (ACTCTTAACCACCCAACCTT) and CMV_R (AACATAGCAGAGATGGCGG) primers were used for the amplification of 280 nt fragment of the coat protein gene of CMV (Faggioli et al., 2005). The PCR conditions were denaturation at 95°C for 3 min followed by amplification as follows: 35 cycles of 30 s at 94°C, 45 s at 55° and 45 s at 72°C with a final extension step at 72°C for 7 min. The RNA extracted from healthy plants was used as negative controls in PCR reactions. Following this, electrophoresis in a 1.5% agarose gel stained with RedSafe™ (iNtRON, USA) was used to visualize the PCR products. After visualization of agarose gel, the amplicons of TSWV and CMV RNAs isolated from tomato and pepper were selected for further analysis.

Phylogenetic analysis

Forward and reverse nucleotide sequences for each selected sample were acquired and edited by the programs Chromas Pro Version 2.5.1., ClustalX 2.00 and software BioEdit. The sequences were submitted to GenBank after nucleotide BLAST (Basic Local Alignment Search Tool) analysis at NCBI (The National Center for Biotechnology Information) and confirmation of sequence identity. Phylogenetic analysis was used to determine the genetic diversity of TSWV and CMV isolates infecting tomato and pepper with isolates from around the world. The nucleotide sequences were aligned with the sequences of TSWV and CMV isolates belong to various countries and hosts retrieved from the GenBank database. Due to the large numbers of identical sequences acquired, phylogenetic trees were drawn using only the selected and then trimmed sequences. Sequence identities were compared using program SMS (The Sequence Manipulation Suite). The phylogenetic trees were constructed in MEGAX (Kumar et al., 2018) using the Maximum Likelihood method and the Tamura-Nei model (Tamura and Nei, 1993), with 1000 bootstrap replicates.

RESULTS

A diagnostic study was done to assess the prevalences of TSWV and CMV in tomato and pepper growing districts of İzmir, Turkey. As seen from the data in Table 1 that, samples were collected in 5 districts encompassing 31 locations. Within the scope of the field studies, tomato and pepper crops showing characteristic virus disease symptoms were collected. The tomato plants collected had leaf curling, stunting, ringspots, chlorotic and necrotic spots. The foliar symptoms of pepper plants were mosaic, ringspots, chlorotic and necrotic spots. Molecular characterization was carried out using RT-PCR with nucleocapsid protein (N) gene-specific primers of TSWV and capsid protein (CP) gene-specific primers of CMV. RT-PCR amplification with specific primers yielded a 777 bp expected fragment of TSWV and a 280 bp of CMV. No viral amplicon was obtained from healthy control plants. The results obtained from the analysis are also summarized in Table 1. The results of this study indicated that the overall samples were infected with TSWV at a rate of 36.98% and with CMV at a rate of 22.94%. TSWV co-infection with CMV was found in 2.15% percent of tomato and 9.81% of pepper samples tested. In summary, these results show that 43.01% of tomato plants and 89.62% of pepper plants were found to be infected with either one of the virus. The results of the current study showed that tomato plants were infected at the same rate (21.50%) with TSWV and CMV. Out of the tested pepper samples, 64.15% were infected with TSWV and 25.47% with CMV (Table 1).

Table 1. Numbers of tomato and pepper samples tested by reverse transcription-polymerase chain reaction (RT-PCR) in the districts of İzmir province and of positives for each virus

Çizelge 1. *İzmir iline ait ilçelerde reverse transcription-polymerase chain reaction (RT-PCR) yöntemi ile test edilen domates ve biber örneklerinin ve pozitif örnek sayıları*

Districts	Numbers of tested tomato samples	RT-PCR		Numbers of tested pepper samples	RT-PCR	
		TSWV	CMV		TSWV	CMV
Ödemiş	18	6	14	10	8	4
Torbalı	117	25	13	11	7	2
Tire	22	0	13	29	16	13
Bayındır	20	2	0	13	8	4
Menderes	9	7	0	43	29	4
Total	186	40	40	106	68	27
Percentage (%)		21.5	21.5		64.15	25.47

In order to confirm the identification of viruses determined by RT-PCR in tomato and pepper plants, nucleotide sequencing was performed for each isolate of TSWV and CMV from each plant group. Partial nucleocapsid protein (N) gene sequences were obtained from selected isolates of TSWV from tomato and pepper leaves. The nucleotide sequences of them were submitted to GenBank under the accession numbers OM517148 and MZ666393, respectively. The nucleotide identity rates of these isolates with TSWV isolates from other parts of the world were compared by BLAST algorithm. The tomato isolate of

TSWV exhibited 98.65% sequence identity with TSWV isolate from pepper plants in Tokat province of Turkey (MW751975). It showed 98.50% identity with peanut isolate of South Korea (MK372883) and tomato isolates of South Korea (MG001348) and France (FR693255) while it displayed 98.35% identity with tomato isolates of Argentina (MK524185) and China (HM594685). Likewise, it showed 98.35% identity with pepper isolates of France (FR693053) and Argentina (MK524182). It has been determined that it has 97.90% similarity with the pepper isolate from Mersin province of Turkey (MW837080) and tobacco isolate of China (MF193425). The pepper TSWV isolate displayed 99.70% identity with tomato isolate from Zonguldak province of Turkey (MZ568848) and 99.40% identity with tomato isolate from Ankara province of Turkey (MZ568840). Similarly, it was 99.10% identical to the pepper isolate of Montenegro (GU339505). It shared 98.96% nucleotide identity with the pepper isolates from Italy (KM096536) and South Korea (HQ267709), both of which are 100% identical. Also, it showed 98.81% similarity with pepper isolate of France (FR693047), 98.51% similarity with tomato isolate of Italy (KM096542) and 98.36% similarity with pepper isolate of Spain (FR693067). Lastly, it exhibited 97.91% identity with tomato isolate of Spain (FR693266) and 96.72% identity with pepper isolate of Tokat province of Turkey (MW751975).

As well as BLAST analysis, phylogenetic trees were constructed using Maximum Likelihood Method to examine genetic diversity among virus isolates. Phylogenetic analysis provided a deeper understanding of virus evolution and genetic interactions. As shown in Figures 1 and 2, the phylogenetic tree of the sequences obtained by amplifying the N gene region of the S RNA segment of the isolates was constructed with the world isolates. The N-gene phylogenetic tree of TSWV isolate from tomato (Figure 1), based on 11 sequences, yielded two subpopulations. First subpopulation included two pepper isolates with the accession numbers of MW751975 and MW837080 that have been identified in Turkey and also other isolates from different countries. The last subpopulation only consisted of our isolate (OM517148).

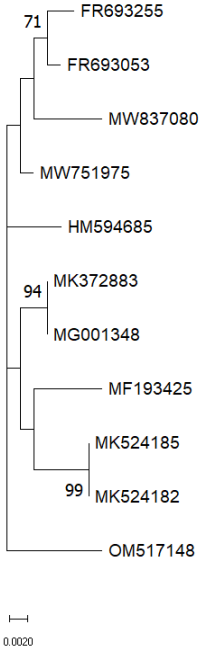

Figure 1. Phylogenetic tree constructed by Maximum Likelihood method using the partial nucleocapsid gene sequences of tomato spotted wilt virus isolates obtained from tomato growing areas in İzmir province and the GenBank database. The bootstrap values are shown in the branches as percentages and values greater than 50% are included in the tree.

Şekil 1. *İzmir domates üretim alanlarından elde edilen ve GenBank veri tabanındaki tomato spotted wilt virus izolatlarının kısmi kapsid protein geni-nükleotit dizileri kullanılarak, Maximum Likelihood yöntemi ile oluşturulan filogenetik ağaç. Bootstrap değerleri dallarda yüzde olarak gösterilmektedir ve %50'den büyük değerler ağaçta yer almaktadır.*

The phylogenetic tree of TSWV isolate from pepper (Figure 2), based on 11 sequences, contained two subpopulations. First subpopulation, which has our isolate (MZ666393), included the isolates reported from Europe and Asia. The last subpopulation consisted one isolate (MW751975) from Tokat province of Turkey.

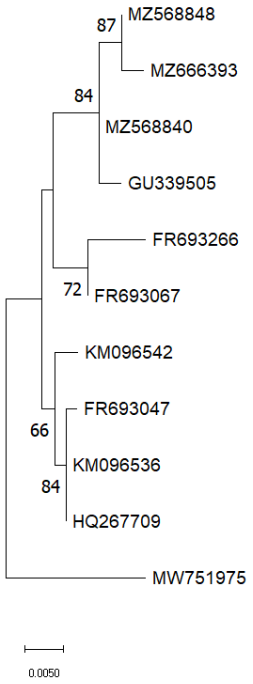

Figure 2. Phylogenetic tree constructed by Maximum Likelihood method using the partial nucleocapsid gene sequences of tomato spotted wilt virus isolates obtained from pepper growing areas in İzmir province and the GenBank database. The bootstrap values are shown in the branches as percentages and values greater than 50% are included in the tree.

Şekil 2. *İzmir biber üretim alanlarından elde edilen ve GenBank veri tabanındaki tomato spotted wilt virus izolatlarının kısmi nükleokapsid protein geni-nükleotit dizileri kullanılarak, Maximum Likelihood yöntemi ile oluşturulan filogenetik ağaç. Bootstrap değerleri dallarda yüzde olarak gösterilmektedir ve %50'den büyük değerler ağaçta yer almaktadır.*

The partial capsid protein (CP) gene sequences of CMV isolates were obtained, and the sequences were deposited to the NCBI GenBank database with the following accession numbers. The accession numbers of the CMV isolates from tomato and pepper crops are MZ666392 and MZ666391, respectively. The nucleotide identity rates of the CMV isolates from tomato and pepper in the present study with the isolates from world were examined. The tomato isolate of CMV was 98.82% identical to the tobacco isolate from Adiyaman province of Turkey (MK704429). Also, a 98.43% identity was determined with tomato isolates from Greece (KT734847) and Iran (KC122260), which were 100% identical. The tomato isolate of CMV shared 98.04% nucleotide similarity with a tomato isolate of Pakistan (MF351527) followed by a cucumber isolate from Iraq (MW477481). Also, it showed 97.65% identity with the bean isolate of Italy (MH748553) and 96.86% identity with the tobacco isolate of India (JX995139). Finally, it has 96.47% similarity with the tomato isolate of China (DQ302718), 96.08% with the pepper isolate of Nigeria (KU976471) and 95.69% with the pepper isolate of Malaysia (HQ107964). The pepper-CMV isolate was 100% identical to the pepper isolate from France (MG334381). Then, a 99.47% identity was found with the pepper isolate of Thailand (AY560556) and the tobacco isolate of Vietnam (HE999617). It shared 98.93% nucleotide similarity with the tomato isolate of Egypt (KX014666) as well as pepper isolates of Tunisia (HE971670), India (KM272276) and Sudan (KU976467). It showed 98.63% identity with the pepper isolate of Malaysia (HQ107960) while it has 98.40% similarity with the pepper isolates of China (FJ403474) and Ecuador (MW291545).

Figures 3 and 4 present the phylogenetic tree of the sequences of RNA3 segment of CMV from tomato and pepper was constructed along with the world isolates. This study showed that the phylogenetic tree of CMV isolate from tomato (Figure 3), based on 11 sequences of the CP gene region, delineated two subpopulations. First subpopulation included European and Asian isolates. The second subpopulation, which has our isolate (MZ666392), consisted of tomato isolates from Asia and Africa. It was also shown that the phylogenetic tree of CMV isolate from pepper (Figure 4), based on 11 sequences, yielded two subpopulations. First subpopulation, which has our isolate (MZ666391), included pepper isolates from France, Tunisia, Malaysia, India, Thailand and Ecuador, as well as, tobacco isolate from Vietnam. The last subpopulation consisted of the isolates from Asia and Africa.

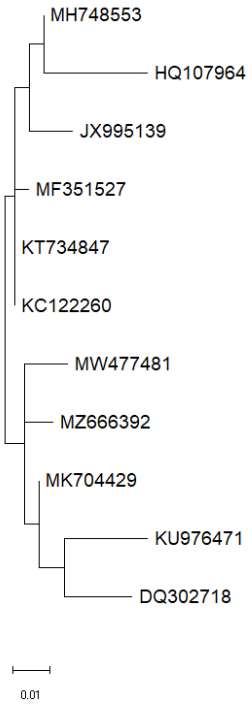

Figure 3. Phylogenetic tree constructed by Maximum Likelihood method using the partial capsid protein gene of cucumber mosaic virus isolates obtained from tomato growing areas in İzmir province and the GenBank database. The bootstrap values are shown in the branches as percentages and values greater than 50% are included in the tree.

Şekil 3. *İzmir domates üretim alanlarından elde edilen ve GenBank veri tabanındaki cucumber mosaic virus izolatlarının kısmi kapsid protein geni-nükleotit dizileri kullanılarak, Maximum Likelihood yöntemi ile oluşturulan filogenetik ağaç. Bootstrap değerleri dallarda yüzde olarak gösterilmektedir ve %50'den büyük değerler ağaçta yer almaktadır.*

The nucleotide identity rates of the CMV isolates from tomato and pepper in the present study with the isolates from world were examined. The tomato isolate (MZ666392) of CMV was 98.82% identical to the tobacco isolate from Adiyaman province of Turkey (MK704429). Also, a 98.43% identity was determined with tomato isolates from Greece (KT734847) and Iran (KC122260), which were 100% identical. The tomato isolate of CMV shared 98.04% nucleotide similarity with a tomato isolate of Pakistan (MF351527) followed by a cucumber isolate from Iraq (MW477481). Also, it showed 97.65% identity with the bean isolate of Italy (MH748553) and 96.86% identity with the tobacco isolate of India (JX995139). Finally, it has 96.47% similarity with the tomato isolate of China (DQ302718), 96.08% with the pepper isolate of Nigeria (KU976471) and 95.69% with the pepper isolate of Malaysia (HQ107964). The pepper-CMV isolate (MZ666391) was 100% identical to the pepper isolate from France (MG334381). A 99.47% identity was found with the pepper isolate of Thailand (AY560556) and the tobacco isolate of Vietnam (HE999617). It shared 98.93% nucleotide similarity with the tomato isolate of Egypt (KX014666) as well

as pepper isolates of Tunisia (HE971670), India (KM272276) and Sudan (KU976467). It showed 98.63% identity with the pepper isolate of Malaysia (HQ107960) while it has 98.40% similarity with the pepper isolates of China (FJ403474) and Ecuador (MW291545).

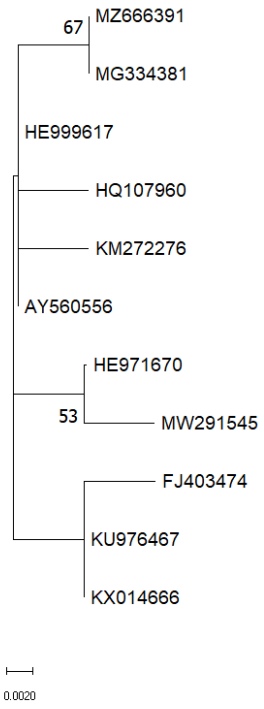

Figure 4. Phylogenetic tree constructed by Maximum Likelihood method using the partial capsid protein gene of cucumber mosaic virus isolates obtained from pepper growing areas in İzmir province and the GenBank database. The bootstrap values are shown in the branches as percentages and values greater than 50% are included in the tree

Şekil 4. *İzmir biber üretim alanlarından elde edilen ve GenBank veri tabanındaki cucumber mosaic virus izolatlarının kısmi kapsid protein geni-nükleotit dizileri kullanılarak, Maximum Likelihood yöntemi ile oluşturulan filogenetik ağaç. Bootstrap değerleri dallarda yüzde olarak gösterilmektedir ve %50'den büyük değerler ağaçta yer almaktadır*

Considering the subgroups of CMV, the sequences of subgroups IA, IB and II from GenBank were selected and included in phylogenetic analysis. The partial CP-gene sequence of CMV isolate (MZ666392) collected from tomato plant showed nucleotide identity (98.43%) with a CMV isolated from melon plant in Iran (KT626606) that is clustered in subgroup IB, characterized by Asian strains. Likewise, CMV isolate (MZ666391) collected from pepper plant showed nucleotide identity (95.72%) with a CMV isolated from melon plant in Iran (KT626606) that is placed into subgroup IB. The phylogenetic trees, as shown in Figure 5, formed one cluster consisting of isolates of subgroup IB along with the CMV isolates of tomato and pepper, whereas the other cluster consisted of isolates of subgroup IA. The isolates belonging to subgroup II formed distinct cluster. Correspondingly, it can be implied that the both CMV-tomato and pepper isolates of this study belong to subgroup IB and have a close phylogenetic relationship with the CMV isolates of subgroup IB found in Iran (KT626606), Nigeria (OK107532) and China (EF213025). According to BLAST analysis of the isolates from different subgroups, CMV-tomato and pepper isolates of the current study exhibited 94.90%-95.19% sequence identities with the CMV isolate from tomato plant in Nigeria (OK107532), and 93.73%-92.51% identities with cabbage isolate from China (EF213025), respectively. However, they showed 91.76%-91.44% identities with zucchini isolate from Poland (KX495208), 91.76%-91.98% with tobacco isolate from Syria (AB448694), and also 90.59%-90.91 identities with banana isolate of Ecuador (KU127245), respectively, which are all classified as subgroup IA. On the other hand, it was determined that CMV-tomato and pepper isolates had nucleotide

similarity ratios of 81.18%-81.28% with pepper isolate of Australia (KU976485), 80.78%-81.28% with tobacco isolate of USA (AJ810256) and 80.00%-80.75% with tobacco isolate of Germany (KU976486), respectively, in subgroup II.

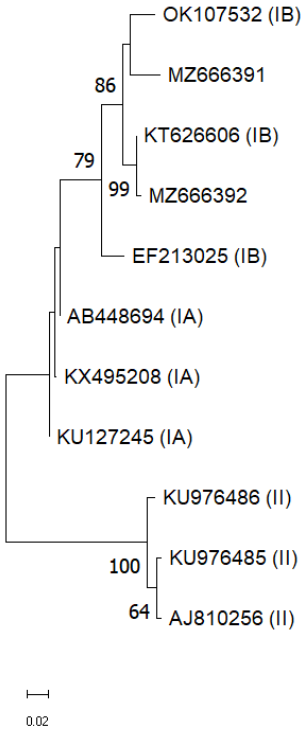

Figure 5. Phylogenetic relationship of cucumber mosaic virus (CMV) isolates of tomato and pepper plants and the isolates of different subgroups of CMV. The bootstrap values are shown in the branches as percentages and values greater than 50% are included in the tree.

Şekil 5. *Domates ve biber bitkilerinden elde edilen cucumber mosaic virus (CMV) izolatlarının, farklı alt gruplardaki CMV izolatları ile filogenetik ilişkisi. Bootstrap değerleri dallarda yüzde olarak gösterilmektedir ve %50'den büyük değerler ağaçta yer almaktadır.*

DISCUSSION

The climatic conditions in Turkey favor tomato and pepper cultivation. This study reported CMV and TSWV infections in the districts of İzmir province. A number of researchers have reported TSWV and CMV infections in tomato and pepper growing areas in different locations of the country. In 1998-1999, serological tests revealed that 20.2% to 7.7% of pepper samples tested were infected with CMV and 2.2% to 9.2% with TSWV in Samsun province (Arli-Sokmen et al., 2005). In 2002-2003, TSWV and CMV were detected 12.9% and 6.9% of the tested tomato crops, respectively, in the same region of Turkey (Arli-Sokmen & Sevik, 2006). CMV was detected in tomato plants in the locations of the north-west Mediterranean region of Turkey by DAS-ELISA, and the infection rate varied from 12.5% to 90.32% (Yardımcı & Eryiğit, 2006). CMV infection in tomato and pepper crops was detected at the rates of 87% and 69%, respectively, using DAS-ELISA and Real-Time PCR in the locations of Marmara Region of Turkey (Uzunoğulları & Gümüş, 2015). In 2015, of the pepper samples tested, 35.81% were found to be infected with TSWV and 7.34% with CMV in the Mediterranean region of Turkey as a result of DAS-ELISA and RT-PCR tests (Güneş & Gümüş, 2019). Although different studies have reported these infectious agents in Turkey, a small-scale study has been carried out in İzmir province (Gümüş, 1998).

This is the study conducted in details so far showing that tomato and pepper samples were infected with notably rate with TSWV and CMV in this region. In spite of the fact that the present findings were consistent with those observed in earlier studies, and the prevalence of viruses was higher in pepper samples (89.62%) than tomato (43.01%). The rates of TSWV and CMV infection may vary depending years in tomato production areas as determined by many researchers using especially biological indexing, DAS-ELISA and RT-PCR. Virus intensity could be variable among geographical locations. In the study carried out in Bulgaria, no Orthotospovirus species other than TSWV were found in tomato crops (Hristova et al., 2001). In 2000-2001, all collected tomato and pepper samples were found to be positive for TSWV in Italy (Roggero et al., 2002). TSWV isolates were identified from tomato and pepper plants grown in provinces in South Korea (Lee et al., 2011). It was determined that TSWV was distributed to different tomato production regions of Kenya with an incidence of 21.81% (Macharia et al., 2015). A considerable amount of literature was published on resistance breaking-TSWV isolates in tomato and pepper cultivation areas in Turkey and Spain (Deligoz et al., 2014; Debreczeni et al., 2015; Fidan & Sari, 2019; Almási et al., 2020). In 2014-2016, CMV was found to be as the major virus among surveyed pepper growing regions in Thailand by ELISA method (Phatsaman et al., 2021). In Rwanda, hot pepper genotypes were evaluated for resistance to CMV under screenhouse conditions by ELISA and RT-PCR, and all commercial genotypes were found to be susceptible (Waweru et al., 2020). CMV infection rate was reported as 64.7% in evaluated chili pepper varieties in Colombia by using molecular techniques (Rivera-Toro et al., 2020). More recently, chili pepper is reported to be affected by CMV with 47.91% infection rate in India (Vinodhini et al., 2021). The present study confirms previous findings and demonstrate that tomato plants have CMV infection rate as similar to pepper plants. Phylogenetic analysis indicated that tomato and pepper isolates of CMV in this study have a close relationship with CMV isolates belonging to subgroup IB (Figure 5). This research may serve as a base for future studies.

It is important to note that the co-infection was detected in tomato and pepper areas surveyed. The mixed infection of TSWV with CMV have been reported previously (Arli-Sokmen & Sevik, 2006). The mixed infection in the host plant allows for virus interactions, resulting in genetic variation. Mixed viral infections can induce more severe symptoms than a single viral infection, or symptoms can be masked by other viruses. Pepper plants involving mixed infection in this study showed severe mosaic and vein banding. The interaction of viruses belonging different genus on a single host during combined infections is needed to be studied.

CONCLUSION

TSWV and CMV were found to cause disease in economically important Solanaceous crops grown in the Aegean region, according to this study. The viruses were found to be more prevalent in pepper production areas than tomato production areas in this location. Despite of being aware of the symptoms of viral infections that affect plants, there was low awareness of the virus epidemiology, specifically their alternative hosts. Therefore, farmers' efforts to control thrips and aphids, as well as eradication of weeds, especially nearby the fields, were limited throughout the vegetation period. This area includes a variety of crops along with weeds, which are alternative hosts for both viruses and their vectors. Overall, weeds could be associated to the long-term presence of viral infections in the area. Therefore, it is important to apply efficient weed management methods for virus control.

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