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RESEARCH ARTICLE

Serologic and molecular determination and phylogenetic analysis of some viruses infecting pepper in Turkey

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ABSTRACT

Viruses are among the major agents causing yield loss in pepper production. During the 2016-2017, a survey was performed in pepper fields to determine of some viruses in pepper plants showing virus-like symptoms in different provinces of Turkey. Plant samples were assayed using DAS-ELISA with specific antisera for cucumber mosaic virus (CMV), tomato spotted wilt virus (TSWV), tomato yellow leaf curl virus (TYLCV), alfalfa mosaic virus (AMV), and pepper mild mottle virus (PMMoV). The DAS-ELISA results indicated that 42.2, 17.5, 17.5, and 10.6 of the 421 samples were infected with CMV, TSWV, AMV, and PMMoV, respectively. The positive samples were further analysed by RT-PCR and sequencing. Based on sequence analyses, a phylogenetic tree was drawn for each of CMV, PMMoV and AMV. Phylogenetic analysis showed that CMV isolates were placed in subgroup IA within the major group I. The AMV isolates were classified within four clades and showed a high variability. PMMoV isolates were grouped with Serbia, Brazil, and Japan isolates within the group I. One AMW isolates determined possible recombinant isolates. Further studies needed for more information about recombinant isolates and evolutions. The analyses of the molecular structure of viruses is important for the accurate diagnosis of the pathogen and to apply the right method of management strategies for controlling plant viruses in agriculture.

Keywords: Alfalfa mosaic virus; Cucumber mosaic virus; Pepper; Pepper mild mottle virus

INTRODUCTION

Turkey is one of the most important pepper producing countries in the world. Pepper (*Capsicum annuum* L.) is the second, most widely cultivated vegetable in Turkey after tomato (TURKSTAT, 2020). Plant viral diseases are economically important and cause important yield reduction in pepper production. Over 100 viral pathogens can infect pepper plants (Kenyon et al., 2014). In addition, yield and quality losses due to virus infections are common in the pepper production. Pepper plants are negatively affected by plant pathogenic viruses in the region. In addition, the high genetic diversity of viral strains complicate their management.

The annual yield losses in pepper caused by pathogenic viruses can sometimes be as high as 100% (Kenyon et al., 2014). Cucumber mosaic virus (CMV) (Nakazono-Nagaoka et al., 2005), Potato virus Y (PVY) (Karasev and Gray 2013), Tomato spotted wilt virus (TSWV) (Arli-Sokmen et al., 2005), Tomato mosaic virus (ToMV) (Gilardi et al., 2004), Tobacco mosaic virus (TMV), Potato virus X (PXV) (Cong et al., 2019), Beet curly top virus (BCTV) (Chen et al., 2011), Pepper yellow leaf curl virus (PYLCV) (Dombrovsky et al., 2010), PMMoV (Genda et al., 2007) and Pepper veinal mottle virus (PVMV) (Fajinmi, 2013) are the most important and common viruses infecting pepper plants. Different researchers have determined TSWV (Arli-Sokmen et al., 2005), CMV (Arli-Sokmen et al., 2005; Uzunogullari and Gumus 2015; Ozdag and Sertkaya, 2017), PVY (Arli-Sokmen et al., 2005), AMV (Arli-Sokmen et al., 2005), and PMMoV (Caglar et al., 2012) on pepper in Turkey based on serological studies.

CMV has a host range of more than 1, 200 species, causes severe losses in vegetables including pepper, and is transmitted by many species of aphids in a nonpersistent manner (Moury and Verdin 2012). Affected pepper plants show different symptoms from mild to severe. Mostly mild mosaic, mottling, vein banding, leaf and fruit deformation,

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stunting symptoms are observed. CMV is a widespread member of the Bromoviridae family. It has three RNA segments composed of single-stranded positive-sense RNAs (RNA 1, 2 and 3). Previous studies reported that CMV can be divided into two subgroups (I and II) based on the sequence variation and serological relationships (Devergne and Cardin, 1973). Furthermore, subgroup I could be further divided into subgroups IA and IB (Jacquemond 2012; Kim et al., 2014). Subgroups IA and II are common worldwide, and subgroup IB is generally found in Asia. Subgroups IA was also reported in Turkey by Kurtoglu and Korkmaz (2018).

AMV, the type species of the genus Alfamovirus in the Bromoviridae family, has a worldwide distribution and infects more than 600 plant species including important crops such as pepper, potato, tomato, tobacco, eggplant, alfalfa, clover, legumes, and woody crops. The pepper plants show distinct bright yellow to white mosaic on leaves, vein necrosis, and chlorotic line patterns when infected with AMV (Kenyon et al., 2014). The virus is spread by aphids in a nonpersistent manner as well as transmitted by seeds in pepper (Kenyon et al., 2014). AMV has a tripartite genome and consists of single-stranded RNA. Based on molecular analysis of coat protein (CP) sequences, Parella et al. (2010) reported that AMV isolates can be separated into two subgroups (I and II) related to their geographic origin. On the other hand, AMV isolates are grouped in four or more different clades based on sequence analyses (Stanković et al., 2014).

TSWV, the member of the family Bunyaviridae, is common viruses in solanaceous crops. The virus is among the current "top ten" plant viruses concerning economic losses worldwide (Scholthof et al., 2011). TSWV particles are isometric and enveloped in a lipoprotein membrane. They have three single-stranded linear RNAs, one of which is negative polarity and the other two have ambisense polarity, associated with a nucleoprotein to form the nucleocapsid (Scholthof et al., 2011; Moury and Verdin 2012). TSWV causes, chloroses, deformation, necrotic spots and concentric necrotic rings (ringspots) on the leaves and fruits.

PMMoV is among the most important viruses of pepper. It causes mosaics and chlorosis, mottling, necrosis, leaf curling of leaves, and deformation of fruits. PMMoV is transmitted seedborne and soilborne. It is also easily transmitted mechanically, during cultivation. The virus belongs to the Tobamovirus genus and has a rod-shaped particle with a positive-sense RNA genome (Fraile and García-Arenal, 2018).

Although various viruses have been reported in different regions of Turkey, limited studies have been conducted, particularly in Tokat province, on the identification and molecular characterization of viruses from pepper fields. In the present study leaf and fruit samples were collected from Tokat, Antalya and Mersin provinces to test for the major pepper viruses using serological and RT-PCR methods. The coat protein (CP) of CMV, AMV, PMMoV were amplified, sequenced and analysed to evaluate molecular relationships with reference isolates from GenBank.

MATERIALS AND METHODS

Plant material

During the mid or late season of years 2016-2017, a total of 421 leaf and fruit pepper samples were collected from symptomatic plants in pepper production regions in different districts of Tokat (n = 320,), Mersin (n= 46,) and Antalya (n= 55,) provinces in Turkey. Samples from Tokat and Mersin provinces were collected from open pepper growing field, while Antalya samples were collected from greenhouse. Collected samples were kept at − 20°C until use.

Serological tests

Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) test was carried out for determining virus infections for CMV, TSWV, AMV, TYLCV and PMMoV on collected samples (Clark and Adams 1977). The test producers were made according to the manufacturer's instructions (Bioreba, Switzerland).

Molecular studies

RNA isolation. For molecular tests, total RNA was extracted according to Astruc et al. (1996). One gram plant tissue was grounded in extraction buffer solution (100 mM Tris-HCl (pH 8.0), 50 mM EDTA, 0.5 M sodium chloride (NaCI) and 0.1% 2-mercaptoethanol with 1: 2 (w/v) and centrifuged at 4,000 g for 5 min and separated the supernatant from the sediment into new sterile tube. A volume of 50 µl of 20% sodium dodecyl sulphate (SDS) was added to tubes, and then the tubes were incubated at 65ºC for 30 min. After adding 250 µl of 5 M potassium acetate (KAc) (pH 6.5), the tubes were transferred on ice for 20 min and centrifuged at 10,000 g for 15 min. The 500 µl supernatant were transferred to a new tube, and an equal amount of 96% ethanol was added to each tube for nucleic acid precipitation and centrifuged at 4,000 g for 5 min. The pellet was resuspended in 100 µl RNAse free sterile water. The resultant RNA was stored at –20°C until used in the two-step RT-PCR tests.

cDNA synthesis and RT-PCR methods

Using 2 µl of total RNA for reverse transcription (RT), complementary DNA (cDNA) synthesis was performed

in a 25 µl volume with hexamer primer for 25ºC for 10 min, followed by 42ºC for one hour and 72ºC for 10 min. PCRs was performed in 25 µ1 of a mix containing 2.5 µl of the cDNA, 5 µL of 5X green reaction buffer, 2 µL of $MgCl_2$ (25 mM), 0.2 µL of dNTPs (25 mM), 0.5 µL of 10 µM of each specific primer, 0.25 µL of Go *Taq* Flexi DNA polymerase (Promega, USA) and 16.5 µl of sterile distilled water. PCR reactions were performed according to references in Table 1. PCR products were analysed on 1.5% agarose gel containing ethidium bromide and visualized with a UV transilluminator.

Sequencing and phylogenetic analysis

The PCR amplicons were sequenced by a commercial company (Life Technologies-Atlas Biotechnologies Lab, Ankara-Turkey) using the Sanger technology. The resultant nucleotide sequences were analysed with ClustalW included in the software MEGAX (Kumar et al., 2018). Maximum-likelihood trees were built with MEGAX, with 1,000 bootstrap replicates.

RESULTS

Surveys of some viruses in pepper plants

Virus-like symptoms were observed all pepper growing areas. During surveys, 421 pepper samples were collected from surveyed areas. Different virus like-symptoms including mosaic, chlorosis, deformation on leaves and fruits, stunting, reduced leaf size, upward curling of leaves without crinkling, severe yellowing of leaves, yellow mottling or patterning on leaves or fruits were observed on sampled plants (Fig. 1). The most common symptoms were mosaic, leaf deformation and stunting of plants. The symptoms could be easily noticed, especially TSWV and AMV infected plants were showed typical symptoms like bright yellow to white mosaic on leaves (Fig. 1a, b), and ringspot on pepper leaves (Fig. 1c). Pepper plants with infected CMV were showed mild mosaic and deformation on the leaves and stunted growth (Fig. 1b).

Occurrence and identification of virus diseases of pepper

DAS-ELISA assays revealed single and mixed infections in the 421 plant samples. In general, according to DAS-ELISA results, 73% of 421 samples collected during the 2016–2017 years were infected by at least one of the viruses. In others (27%), the tested viruses were not found or could not be determined because the virus concentration was low. These samples may be infected with other viruses that were not tested, or symptoms caused by phytotoxicity, nutrient deficiency, or infections of phytoplasma. Based on DAS-ELISA results, CMV was the most common viral pathogen (42.2%) detected in pepper samples from the surveyed areas, followed by the AMV and TSWV (17.5%), and PMMoV (10.6%) (Table 2). CMV was mostly detected in the region of Tokat and Mersin, while TSWV was more widespread in Antalya.

TSWV infections were detected in Antalya province. None of the pepper samples collected from the Antalya province were infected by CMV, AMV, PMMoV or TYLCV.

Table 1: The information of primers used in this study

Primer	Base	Reference
TSWV F	5′-AACCTGCAGCTGCTTTCAAGCAAGTTC-3′	Maiss et al. (1991)
TSWV R	5′-ACAACTTTTAGGATCCTCATGTCTAAGGTT-3′	
CMV F	5′-ACTCCAACTGGCTCGTATGG-3′	Nakazono-Nagaoka et al. (2005)
CMV R	5′-CGCCCTGCAGTGGTCTCCTTTTGGAG-3′	
AMV coat-F	5′-GTGGTGGGAAAGCTGGTAAA-3′	Martínez-Priego et al. (2004)
AMV coat-R	5′-CACCCAGTGGAGGTCAGCATT-3′	
F1- TYLCV	5′-GGAGGAAGGTCRAGCAACAGC-3′	Dombrovsky et al. (2010)
R2-TYLCV	5′-CTATTTGG GGTTGTGYARTTGCAC-3′	
PMMoV P12/3	5′-ACAGCGTTTGGATCTTAGTAT-3′	Valesco et al. (2002)
PMMoV P12/3A	5′-GTGCGGTCTTAATAACCTCA-3′	

F – forward, R – reverse, A – sense, AS – antisense

Fig 1. *Alfalfa mosaic virus* (a), *Cucumber mosaic virus* (b) and *Tomato spotted wilt virus* (c)

In Mersin, CMV, TSWV and PMMoV infections were detected. However, single infection of PMMoV were not detected in tested samples. All PMMoV infected samples from Mersin province were in mixed infection with CMV or TSWV (Table 2).

Moreover, double and triple mixed viral infections were detected in 80 (19%) out of the total samples. Mixed infections of CMV+AMV were the most detected in samples with a frequency of 9.26%. Double infections with PMMoV+CMV, TSWV+CMV, PMMoV+TSWV were accounted for 3.8%, 0.7%, 0.7%, respectively. Triple infections of CMV+AMV+PMMoV and TSWV+AMV+PMMoV were detected in low rates as respectively 0.2% and 0.2% (Table 3). No TYLCV was detected in samples with both DAS-ELISA and PCR tests.

Molecular studies and sequencing

Analysis of the diversity of CMV, AMV and PMMoV isolates was performed mostly with some isolates from Tokat (Table 3) where virus infection rates were the highest. The sequences of CP regions of AMV isolates, RNA 2 segments of CMV isolates and RdRP regions of PMMoV isolates on the genome of Turkish isolates were obtained and their accession numbers were obtained from NCBI and submitted to the GenBank.

Phylogenetic analysis of Turkish CMV pepper isolates

Partial RNA2 segments of eight Turkish CMV isolates were sequenced and deposited in GenBank under accession Nos: MW092071 (TB1), MW092072 (TB5), MW092073 (KLB21), MW092074 (KC5), MW092075 (AC9), MW092076 (AC22), MW092077 (AC29) and MW092078 (AC31). A maximum likelihood tree for a total of 23 CMV isolates was constructed using the partial nucleotide sequences of RNA2 segments. As already known, worldwide isolates were divided into two major groups as I and II, and major group I clustered into two subgroup (subgroup IA and IB). Turkish CMV isolates along with isolates from Iran, South Korea and Japan were placed in subgroup IA. Six Turkish CMV isolates (AC22, AC9, AC29, AC31, TB1 and TB5) clustered together within subgroup IA. Isolate KC5 (MW092074) clustered with Japan, South Korea and Poland isolates and KLB21 (MW092073) isolates showed different clustering within subgroup IA with Iran isolates (Fig. 2). The Turkish CMV isolates showed 94-96% nucleotide and amino acid similarity with LC066502 (Turkey, 2007) and Iran (LC066466, M782239) isolates.

Table 2: Occurrence of virus infections on pepper in the surveyed provinces

Province	Total samples	CMV	TSWV	AMV	PMMoV	TYLCV
Tokat	320	150	41	74	38	-
Antalya	55	-	28	-	-	-
Mersin	46	28	5	-	7	-
Total (Rate)	421	178 (42.2%)	74 (17.5%)	74 (17.5%)	45 (10.6%)	

Phylogenetic analysis of Turkish AMV pepper isolates

AMV was the second most common virus in the pepper fields sampled. Eight AMV isolates positive in DAS-ELISA were selected for RT-PCR and sequenced (accession numbers: MT671388 (ESB2), MT671389 (ESB5), MT671390 (ESB12), MT671391 (ESB13), MT671392 (AC35), MT671393 (AC21), MT671394 (DB1) and MT671395 (BYB14).) A phylogenetic analysis was done based upon the partial CP sequences of the eight AMV isolates from Turkey and 28 reference isolates from GenBank using the Maximum Likelihood tree model (Fig. 3). Based on the phylogenetic analysis, all AMV strains clustered in two monophyletic groups (Subgroup I and Subgroup II). Subgroup I contained Iranian, Canadian and Serbian isolates, whereas subgroup II contained strains from France and England. In the phylogenetic tree, two isolates MT671390 and MT671395 (ESB12 and BYB14) showed high similarity with Italian, Chinese and Serbian AMV isolates. MT671389 (ESB 5) AMV isolate was clustered together with Iranian isolates in the phylogenetic tree. The MT671393 (AC21) and MT671388 (ESB 2) isolates were in the same cluster. MT671394(DB1) isolate showed similar clustering with Canadian isolates. The MT671391 (ESB13) isolate showed different clustering from other Turkish AMV isolates and MT671391 (ESB13) was clustered with France and England isolates in subgroup II (Fig. 3).

Phylogenetic analysis of Turkish PMMoV pepper isolates

Four Turkish PMMoV isolates were sequenced and submitted to GenBank with the accession numbers: MT671372 (CS1), MT671373 (CS4), MT671374 (DB18) and MT671375 (DB22). According to phylogenetic tree analysis based upon RNA-dependent RNA polymerase (RdRp) gene of PMMoV, the isolates were divided into two

Table 3: Occurrence of mixed infections of the viruses on pepper

	TSWV+CMV	CMV+PMMoV	PMMoV+TSWV	CMV+AMV	CMV+AMV+PMMoV	TSWV+AMV+PMMoV
Tokat	1	10	2	39	1	1
Mersin	2	6	1	-	-	-
Antalya	-	-	-	-	-	-
Total	3	16	3	39	1	1

Fig 2. Maximum likelihood tree of CMV partial 539 nt sequences of CP region. Accession numbers of each isolate are indicated in the tree. The scale bar represents a genetic distance of 0.05. Bootstrap values above 70% (n= 1000 bootstraps) are indicated for each node. Sequences obtained in this study are in bold.

groups. All four Turkish PMMoV isolates were clustered into Group I including references isolates from China, Spain, India and South Korea (Fig. 4). Turkish PMMoV isolates were showed 98-99% sequence identity with isolates from China, Brazil, India isolates.

DISCUSSION

This study allowed to determine and characterize the viruses infecting pepper, and to determine the genetic variability among CMV, AMV, PVY and PMMoV isolates in pepper plant from Turkey. For this purpose, the relative incidences of some viral diseases in pepper cultivation areas in Turkey were determined by DAS-ELISA and RT-PCR methods. Pepper is an economically important crop in Turkey and incidences of virus diseases have also significantly increased in pepper production areas. In surveys, different virus-like symptoms such as mosaic, chlorosis, upward curling of leaves without crinkling, yellow mottling or patterning on leaves or fruits were observed on sampled plants in different provinces (Fig. 1). Typical symptoms of TSVW and AMV were observed on plants in the fields (Fig. 1). The symptoms observed in this study were reported in those previously reported in virus-infected cucurbit fields worldwide (Nakazono-Nagaoka et al., 2005; Karasev and Gray 2013; Gilardi et al., 2004; Cong et al., 2019; Chen

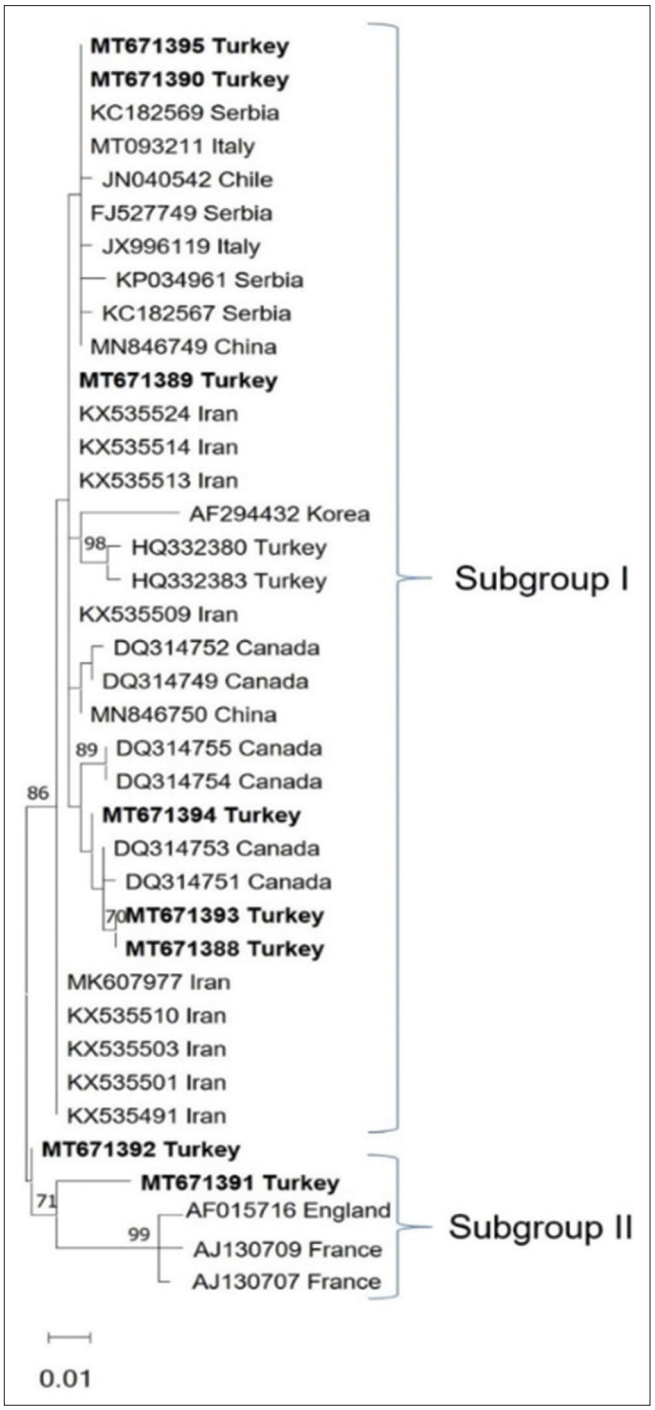

Fig 3. Maximum likelihood tree of AMV partial 333 nt sequences of CP region. Accession numbers of each isolate are indicated in the tree. The scale bar represents a genetic distance of 0.01. Bootstrap supports above 70% (n=100 bootstraps) are indicated for each node. Sequences obtained in this study are in bold.

et al., 2011). The occurrence and incidence of viruses on pepper plants have been determined in different studies in Turkey. The infections of CMV, PVY, AMV and PMMov have been reported in previous studies (Arli-Sokmen et al 2005; Uzunogullari and Gumus, 2015; Ozdag and Sertkaya 2017); Caglar et al., 2012). Ozdag and Sertkaya (2017) also

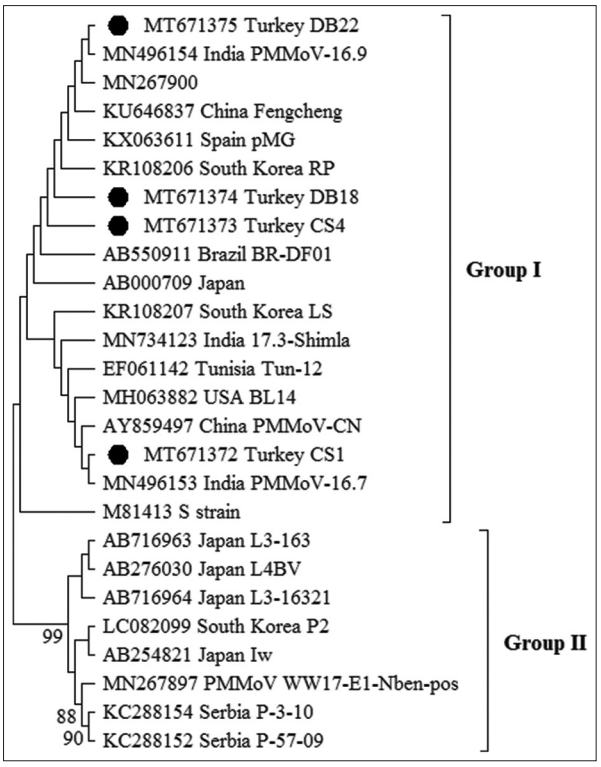

Fig 4. Maximum likelihood tree of PMMoV partial 762 nt sequences of RdRP region. Accession numbers of each isolate are indicated in the tree. The scale bar represents a genetic distance of 0.01. Bootstrap supports above 70% (n=100 bootstraps) are indicated for each node. Sequences obtained in this study are in bold. The scale bar represents a genetic distance of 0.05.

reported that similar symptoms as stunting, chlorosis, yellow patterning on leaves or fruits were observed pepper plants in Hatay provinces.

The highest incidence rate was obtained in Tokat province, and the lowest relative incidence rate was obtained in the Antalya province. Tokat and Mersin samples were collected from open fields, while Antalya samples were collected from the greenhouse. Since greenhouse conditions are more controlled environments, the spread of viruses is restricted, what can explain the lower infection rate in Antalya in this survey. In the present study, CMV and AMV were identified as the most prevalent viruses on pepper in Tokat province. They are both effectively transmitted by insect vectors, especially aphids (Hemiptera). The vegetables are grown in open fields in Tokat and Mersin provinces, which may stimulate the spread of viruses in the province. CMV was the most commonly observed virus in the current study in both Tokat and Mersin provinces, whereas AMV was detected only in Tokat province especially more intensely in pepper plantations that are close to alfalfa fields. No TYLCV positive samples was detected in surveyed area.

According to phylogenetic analysis, the Turkish CMV isolates all belong to subgroup IA. When nucleotide variations were detected throughout the RNA2 sequences, important variations were observed on the 5' and 3' untranslated regions of RNA2 as reported by Kim et al. (2014). Jacquemond (2012) analysed the population genetics of CMV and emphasized the significance of analysing sequences of full genome segments of CMV. So far, a lot of sequence information using complete or partial genomic sequences have been reported about CMV from different parts of the world (Kim et al., 2014), and indicate that reassortment between the different RNAs has taken place during the evolution of the virus (Ohshima et al., 2016). CMV were clustered three molecular group as IA, IB, and II based on the information about different regions of the genome (Eiras et al., 2004). In previous studies in Turkey, CMV isolates have been determined in different plant hosts (Ergun et al., 2013; Karanfil and Korkmaz 2017; Guneş and Gumuş 2019; Guller and Usta 2020; Yeşil 2020). Most of the Turkish CMV isolates fell into the subgroup IA as the Turkish CMV isolates. Two CMV isolates from cowpea and tobacco crops in Turkey were classified into the subgroup IB (Karanfil and Korkmaz 2017; Guller and Usta 2020). Further sequence data are needed to characterize the population structure of CMV from Turkey and other countries.

For AMV, the isolates collected from the same region in Turkey showed different clustering among themselves (Fig. 3). Different authors reported different clustering of the AMV isolates (Stanković et al., 2014; Oreshkovikj et al., 2017). AMV isolates were separated into two groups of I and II by Parrella et al. (2010). Parrella et al. (2011) further divided the second group as IIA and IIB subgroups. Subsequently, Stankovıć et al. (2014) grouped the AMV isolates in four or more different groups based on sequence information of coat protein region. In this study, Turkish AMV isolates were grouped similarly as reported by Stankovıć et al. (2014) and Oreshkovikj et al. (2017). Moreover, AMV isolates analysed in this study had different clustering from Turkish AMV isolates (HQ332380, HQ332383) which were deposited in GenBank. In order to determine, if ESB13 (MT671391) is a recombinant derived from viruses belonging to the two main subgroups, a search for recombination was carried out by using RDP, SISCAN, BOOTSCAN, PHYLPRO, GENECONV, MAXCHİ, CHIMAERA, 3SEQ implemented in RDP4 program. According to the presence of recombination events (positions 166-281). The potential major parent is HQ332383 from Turkey, and the minor parent is unknown. These results indicate that ESB13 is possible recombinant AMV isolate.

L genes L^1, L^{1a}, L^2, L^3 and L^4 in *Capsicum* spp. confer resistance to Tobamoviruses. Gilardi et al. (2004) reported that the S strain of PMMoV (PMMoV-S; P1,2 pathotype)

elicits the L^3 gene, and PMMoV-I (P1,2,3 pathotypes) elicits a response from the L^4 gene. Genda et al. (2007) reported that $P_{1,2,3,4}$ is the first pathotype to able to overcome the resistance conferred by the L^4 gene in *Capsicum* spp. Genda et al., (2007) also found that a double amino acid change is needed in the coat protein region of L4BV isolate to overcome L^4 gene resistance. In Turkey, PMMoV is presumably biologically identical to pathotype $P_{1,2,3}$ (Caglar et al., 2012). Caglar et al. (2012) reported that based on biological tests or molecular analyses, Turkish PMMoV isolates clustered with PMMoV pathotypes $P_{1,2,3}$ isolates from Italy, Spain and Israel, all of which were reported to have overcome the L^3-resistance gene in pepper. Subsequently, Fidan and Barut (2019) sequenced one isolate from Antalya province that belonged to pathotypes $P_{1.2}$ PMMoV was sparsely observed in the present study. However, this virus is easily transmitted in mechanical ways like contact way or cutting of old leaves. In this study, RdRp regions of PMMoV isolates were sequenced, and studies on the CP region and/or biological tests are needed to determine if they can break some resistance genes in pepper.

CONCLUSION

Viral diseases in particular are one of the main limiting factors in pepper production. In this study, some pepper viruses (CMV, AMV, PMMoV) have been determined using serologic and molecular methods. Later phylogenetic trees were obtained by performing sequence analysis of the samples belonging to three different viruses that were positive, and the degree of relatedness was determined by comparing them with reference isolates. In the study, one AMW isolates determined possible recombinant isolates. Further studies needed for more information about recombinant isolates and evolutions. Therefore, analysing the molecular structure of viruses is very important for the accurate diagnosis of the pathogen and to apply the right method of management strategies for controlling plant viruses.

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