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Detection and Characterization of *Tomato spotted wilt virus* and *Cucumber mosaic virus* on Pepper Growing Areas in Antalya

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ARTICLE INFO

Research Article DOI: 10.15832/ankutbd.499144
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ABSTRACT

The most efficient method to control the plant virus diseases is breeding resistant cultivars. However, the resistance could be broken down after using resistant cultivars. This study was aimed to determine the prevalence and also serological and molecular characterization of *Tomato spotted wilt virus* (TSWV) and *Cucumber mosaic virus* (CMV) that cause infections, especially, in resistant pepper cultivars. For this reason, samples were collected from pepper growing greenhouses and open fields during vegetation period of 2015 in different parts of Antalya province including Kumluca, Demre, Serik and Aksu districts. Out of 148 pepper samples collected, 53 (35.81%) were infected with TSWV and 11 (7.34%) with CMV as a result of Double-Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) test. Some regions on S RNA (nucleocapsid protein gene), M RNA (glycoprotein gene) and L RNA (RNA-dependent RNA polymerase gene) of TSWV genome; RNA 1 (helicase/methyltransferase gene) and RNA 3 (coat protein gene) of CMV genome of DAS-ELISA positive samples were amplified by RT-PCR with specific primers. Nucleotide similarity rates of nucleocapsid protein gene, glycoprotein gene and RNA-dependent RNA polymerase gene regions of TSWV isolate varied between 92-98% identity with other isolates in GenBank and CMV isolate varied between 89-96%. TSWV isolate showed nucleotide identity varied between 92-97% with *Tsw* resistance breaking isolates from Capsicum plants in Turkey, Italy and Spain. Genetic determinant for overcoming pepper *Tsw* resistance is located in S segment but aminoacid substitutions responsible for TSWV breakdown remain contradictory in several reports.

Keywords: *Cucumber mosaic virus* (CMV); *Tomato spotted wilt virus* (TSWV); DAS-ELISA; RT-PCR; Detection; Characterization

1. Introduction

TSWV (*Tomato spotted wilt virus*), type member of the genus Orthotospovirus within the Bunyaviridae family, first reported on capsicum in Turkey by Yurtmen et al (1999), is rated among the ten most economically devastating plant viruses worldwide (Adkins 2000). TSWV is transmitted persistently through several species of thrips especially *Frankliniella occidentalis* (Mound 2001) and one of the major problem in pepper production areas in Antalya province of Turkey. The genome of TSWV consists of three negative or ambisense single-stranded RNAs designated as L (large), M (medium) and S (small) (Adkins 2000). The most efficient method to control the virus in pepper crops is breeding resistant cultivars harboring the pepper

resistance gene *Tsw* toward TSWV. However, the resistance gene *Tsw* has broken down in several Mediterranean countries after using resistant cultivars (Roggero et al 2002; Margaria et al 2004). Antalya is one the province in Turkey where the most resistant pepper cultivars against TSWV disease are being cultivated. Even though it has been reported that *Tsw* has broken down in Samsun province of Turkey (Deligoz et al 2014), there is no record about *Tsw* resistance in pepper production areas of Antalya province.

One of the most prevalent viral constraint that affecting pepper production is CMV (*Cucumber mosaic virus*), type member of the genus Cucumovirus within the Bromoviridae family (Moury & Verdin 2012), was found on pepper growing areas in Turkey first by Yılmaz & Davis (1985). It was detected in pepper plants at different rates in various provinces in Turkey by DAS-ELISA mostly (Arli-Sokmen et al 2005; Buzkan et al 2006; Uzunoğulları & Gümüş 2015). CMV transmitted by more than 75 aphid species in non-persistent manner especially by *Myzus persicae* and *Aphis gossypii* (Perry et al 1998). The genome of CMV consists of three positive-sense single stranded RNAs designated as RNA 1, RNA 2 and RNA 3 (Kumari et al 2013). It has been reported that CMV resistant hot pepper cultivars which belongs pathotype 0 (P0) showed severe mosaic symptom in Korea and their causal agent was identified as CMV. Pepper isolate of CMV was described as P0 resistance breaking virus. The result suggests that CA-P1-CMV isolate can overcome P0 resistant pepper cultivars (Lee et al 2006).

This study was initiated to determine the prevalence of TSWV and CMV infection especially on resistant pepper cultivars and investigation whether TSWV resistance breaking isolates emerged or not in intensively pepper growing areas of Antalya province of Turkey. Here we report the serological and molecular characterization of TSWV and CMV isolates collected from peppers. The PCR products of nucleocapsid protein gene (S RNA segment), glycoprotein gene (M RNA segment) and RNA-dependent RNA polymerase gene (L RNA segment) regions of TSWV isolate and helicase/methyltransferase gene (RNA 1 segment) and coat protein gene (RNA 3 segment) regions of CMV isolate were sequenced and phylogenetic trees constructed based on nucleotide homology.

2. Material and Methods

2.1. Surveys and collection of virus infected samples

Surveys were conducted in randomly selected pepper growing greenhouses and open fields in Antalya province including Kumluca, Demre, Serik and Aksu districts. The samples was collected from the virus-like symptom showing pepper cultivars during vegetation period in spring and summer of 2015. All samples were stored at -20 °C before testing.

2.2. Enzyme linked immunosorbent assay

DAS-ELISA was performed for detection of TSWV and CMV in the collected plant samples. Specific antibodies for TSWV and CMV were applied according to manufacturer's instruction (Bioreba Switzerland).

2.3. Total RNA extraction, reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was extracted from TSWV or CMV positive samples determined by DAS-ELISA using a previously reported method by Foissac et al (2001) as recommended. RNA extracts were used as template for reverse transcriptase-polymerase chain reaction (RT-PCR). The cDNA synthesis was carried out using 10 µL RNA with 2.0 µL random primers, 0.8 µL of 100 mM dNTP mix, 1.0 µL of RNase inhibitor, 2.0 µL of 10x RT buffer, 1.0 µL of SuperScript™ reverse transcriptase and 3.2 µL Nuclease-free H_2O (Thermo Scientific, USA) in a total reaction mixture of 20 µL. The reaction mixture was incubated at 25 °C for 10 min and 37 °C for 120 min followed by incubation at 85 °C for 5 min. Polymerase chain reaction (PCR) was carried out using 2 µL of cDNA with 25 µL Dream Taq Green PCR Master Mix (Thermo Scientific, USA),

0.5 µL of 10 µmolar forward primer, 0.5 µL of 10 µmolar reverse primer, and 22 µL nuclease free water (Applied Biosystems, USA) in a reaction mix of 50 µL. The PCR amplification was performed in an automated termal-cycler (Gene Amp PCR 9700 systems, Applied Biosystems, USA). Primer sets are mentioned in Table 1. PCR products were electrophoresed in 1% agarose and stained with ethidium bromide.

2.4. Sequencing and sequence analysis

One positive sample was randomly selected for each TSWV and CMV isolate and their amplicons for each segment was sequenced. Nucleotide consensus sequences were assembled and edited using Chromas Pro Version 2.5.1. The sequences were analyzed by NCBI-BLAST analysis. After confirming the identity of the sequences, they were submitted to GenBank. The nucleotide sequences were aligned with 20 other isolates from different countries and hosts. Sequence identity were compared and phylogenetic trees constructed using CLC RNA Workbench Version 6.8.2 (CLC Bio, Denmark) and Vector NTI Software Programme (Invitrogen, USA).

3. Results

3.1. Survey and collection of virus infected samples

To collect virus-like symptoms showing pepper plants 62 samples from Kumluca, 42 samples from Demre, 26 samples from Serik and 18 samples from Aksu districts were collected. The samples were showing stunting, yellowing, leaf deformation and curly top symptoms. Foliar symptoms of samples were chlorosis, ringspots, mosaic, mottling, vein clearing, reduction, curling, chlorotic and necrotic spots. Pepper fruits of samples were showing reduction, ringspot, chlorotic spots and roughness. Symptoms were showed in Figure 1.

3.2. Enzyme linked immunosorbent assay

Out of 148 plants, 11 plants were positive for CMV with overall incidence 7.34% and 53 samples were infected with TSWV with an overall incidence 35.81%. The number of CMV positive samples from Demre was 8 and Serik was 3, the incidence was found 19.04% and 11.53%, respectively. There was no CMV positive samples from Kumluca and Aksu districts. The number of TSWV positive samples was 12 from Kumluca, 10 from Demre, 14 from Serik and 17 from Aksu. The TSWV incidence was found in these districts were 19.35%, 23.80%, 53.84% and 94.44%, respectively. The ELISA results are mentioned in Table 2 and Table 3.

3.3. Reverse transcriptase-polymerase chain reaction (RT-PCR)

Nucleocapsid protein gene, glycoprotein gene and RNA-dependent RNA polymerase gene regions on S RNA, M RNA and L RNA segments of TSWV and helicase/methyltransferase gene and coat

Table 1- Primer sets used for RT-PCR

Primer	*Sequence (5'-3')*	*Region*	*Reference*
TSWV S RNA	F ATGTCTAAGGTTAAGCTCAC R TTAAGCAAGTTCTGC GAGTT	Nucleocapsid protein gene	Nour et al (2013)
TSWV M RNA	F TGCTCACCATCCAACATTTC R CGAGAAGAAGAATCAACCATCC	Glycoprotein gene	Designed by author
TSWV L RNA	F TGTCAAAATCACTGCCGATG R TTCCCCAAAACCCTGCTACT	RNA-dependent RNA polymerase gene	Designed by author
CMV RNA 1	F TCGTTTGACATGCGTTTCTC R TTTAGCCGTAAGCTGGATGG	Helicase/methyltransferase gene	Designed by author
CMV RNA 3	F GTAGACATCTGTGACGCGA R GCGCGAAACAAGCTTCTTATC	Coat protein gene	De Blas et al (1994)

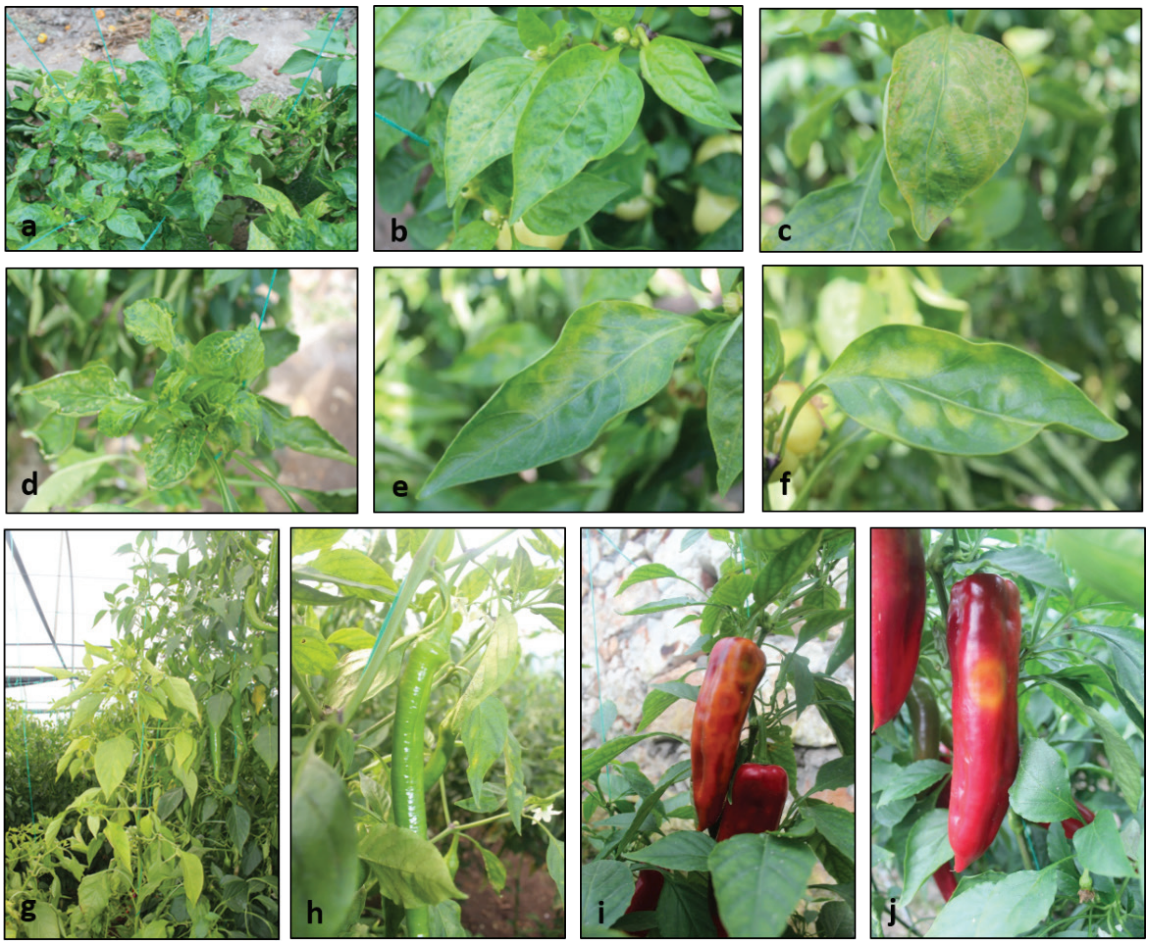

Figure 1- Foliar and fruit symptoms of samples collected from Kumluca and Demre districts. a, Banana pepper, roughness and chlorotic spots; b, Banana pepper, mosaic; c, Sweet bell pepper, chlorotic and necrotic spots; d, Sweet bell pepper, curly top and mottling; e, Sweet bell pepper, chloroticring spots; f, Sweet bell pepper, mottling; g, Long green pepper, stunting and yellowing; h, Long green pepper, chlorotic spots as line shaped; i, Capia pepper, ringspots; j, Capia pepper, chlorotic ringspots

Table 2- The rate of infection with TSWV according to the districts

District	*Number of tested samples*	*Number of infected samples*	*Number of healthy samples*	*Rate of infection (%)*
Kumluca	62	12	50	19.35
Demre	42	10	32	23.80
Serik	26	14	12	53.84
Aksu	18	17	1	94.44
Total	148	53	105	35.81

Table 3- The rate of infection with CMV according to the districts

District	*Number of tested samples*	*Number of infected samples*	*Number of healthy samples*	*Rate of infection (%)*
Kumluca	62	0	62	0.00
Demre	42	8	34	19.04
Serik	26	3	23	11.53
Aksu	18	0	18	0.00
Total	148	11	137	7.34

protein gene regions on RNA 1 and RNA 3 segment of CMV were amplified by RT-PCR with specific primer pairs for molecular characterization (Figure 2 and 3).

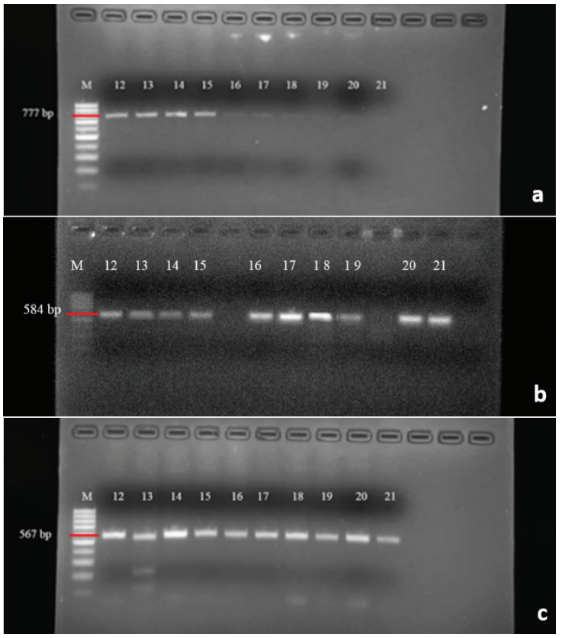

Figure 2- Detection of TSWV by RT-PCR. a, Amplification of T SWV genome with specific primer pair for S RNA segment. DNA marker (M), PCR products: 12–21 (Aksu isolates); b, Amplification of TSWV genome with specific primer pair for M RNA segment. DNA marker (M), PCR products: 12–21 (Aksu isolates); c, Amplification of TSWV genome with specific primer pair for L RNA segment. DNA marker (M), PCR products: 12-21 (Aksu isolates). Isolate number 14 is TSWV Aksu isolate

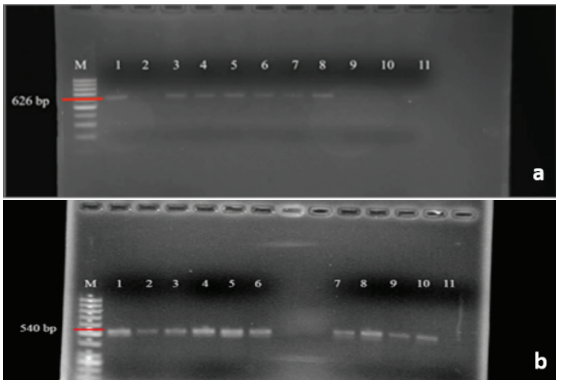

Figure 3- Detection of CMV by RT-PCR. a, Amplification of CMV genome with specific primer pair for RNA 1 segment. DNA marker (M), PCR products: 1-8 (Demre isolates), 9-11 (Serik isolates); b, Amplification of CMV genome with specific primer pair for RNA 3 segment. DNA marker (M), PCR products: 1-8 (Demre isolates), 9-11 (Serik isolates). Isolate number 8 is CMV Demre isolate

3.4. Sequencing and sequence analysis

Molecular characterization was performed for each segment of TSWV and CMV isolates thereafter sequenced. The PCR products for different segments of TSWV Aksu and CMV Demre isolates were sequenced. The sequences of PCR products were submitted to NCBI under accession numbers KY973676 (partial sequence of RNA 3 of CMV isolate), KY973677 (partial sequence of L RNA of TSWV isolate), KY973678 (partial sequence of RNA 1 of CMV isolate), KY973679 (partial sequence of S RNA of TSWV isolate) and KY973680 (partial sequence of M RNA of TSWV

isolate). The sequences obtained by amplification of the region of S RNA, M RNA, L RNA segment of TSWV isolate and RNA 1, RNA 3 segment of CMV isolate was compared with other 20 isolates from all over the world by NCBI-BLAST. The features of isolates from world mentioned in Table 4 and Table 5. Phylogenetic relationships were determined. The Aksu-TSWV isolate S RNA shared 96-98% nucleotide identity and phylogenetic tree was constructed with these isolates (Figure 4). The isolate shared 98% homology with Italy-pepper isolate (GU369722), South Korea-pepper isolate (HQ267713) and New Zealand-chrysanthemum isolate (KC494495). It shared 97% homology with tomato isolates of Samsun (KT192623), New Zealand (KC494501), France (FR693058) and pepper isolates of Antalya (KM407603), France (FR693046), Italy (DQ431238). It also showed 97% homology with *Tsw* resistance breaking (RB) isolates from Capsicum plants from Samsun, Turkey (KM379141) and Italy (DQ431237). It showed 96% homology with Hungary-pepper isolate (KJ649612) and non-resistance breaking pepper isolate (KM379142) from Samsun, Turkey. The Aksu-TSWV isolate M RNA shared 92-97% nucleotide identity and phylogenetic tree was constructed with these isolates (Figure 5). The isolate shared highest homology (97% nt) with USA-dahlia isolate (AY744486). It shared 96% nucleotide identity with South Korea-lettuce isolate (KC261966), Australia-pepper isolate (KT717692), Sw-5 resistance breaking (HM015520) and Sw-5, *Tsw* non-resistance breaking (HM015512) tomato isolates from Spain. It also showed 96% homology with resistance breaking pepper isolate from Italy (HQ830185) while showed 95% homology with Australia-tomato isolate (KM365065). It shared 92% nucleotide identity with *Tsw* resistance breaking pepper isolate from Spain (KP008133) and pepper isolates of Italy (KJ575621), USA (AY744489, KT160281), South Korea (KC261948, KC261957) and China (KM657119). It also showed 92% homology with South Korea-tomato isolate (KC261969), South Korea-chrysanthemum isolate (KC261975), USA-tobacco isolate (AY744490), USA chrysanthemum isolate (AY744483), China-lettuce isolate (JN664253) and China-tomato isolate (JF960236). The Aksu-TSWV isolate L RNA shared 92-98% nucleotide identity and phylogenetic tree was constructed with these isolates (Figure 6). The isolate shared highest homology (98% nt) with New Zealand-tomato isolate (KC494520) and Sw-5 resistance breaking tomato isolate (KP008130) from Spain. It shared 97% nucleotide identity with Italy-pepper isolate (KJ575619), New Zealand-pepper isolate (KC494508), South Korea-lettuce isolate (KC261965) and wilt type tomato isolate (KP008128) from Spain. It showed 96% homology with South Korea-pepper isolates (KC261947, KC261956), Australia-pepper isolate (KT717691), Australia-tomato isolate (KM365064) and South Korea-tomato isolates (HM581934, KC261968). It showed 95% homology with pepper isolate (KM657122) and tomato isolate (JF960237) of China while showed 94% homology with *Tsw*-resistance breaking pepper isolate (KP008132) from Spain, China-tobacco isolate (KM657121) and China-lettuce isolate (JN664254). It showed 93% homology with South Korea-chrysanthemum isolate (KC261974) and South Korea-pepper isolate (HM581937) while showed 92% homology with USA-pepper isolate (KT160280). The Demre-CMV isolate RNA 1 shared 89-96% nucleotide identity and phylogenetic tree was constructed with these isolates (Figure 7). The isolate shared highest homology (96% nt) with Egypt-tomato isolate (KT921314) and 95% homology with Malaysia-cucumber isolate (JN054636). It shared 94% nucleotide identity with India-pepper isolate (KM272277) while 92% with Italy-pepper isolate (HE962478) and India-banana isolate (EU159528). It showed 91% homology with South Korea-potato isolate (KM047509), South Korea-corn isolate (JN180309) and Japan-spinach isolate (LC066420) while showed 90% homology with South Korea-pepper isolates (KC527784, KC527785, KC527787, KC527794), China-tomato isolate (EF216866) and USA-bean isolate (HF572914). It showed 89% homology with South Korea-pepper isolate (KC527789), Japan-cucumber isolate (AB188231), China-chinese cabbage isolate (EF213023) and tomato isolates of France (HE793683), Spain (AM183117) and Japan

(AB368499). The Demre-CMV isolate RNA 3 shared 89-95% nucleotide identity and phylogenetic tree was constructed with these isolates (Figure 8). The isolate shared 95% homology with Malaysia-cucumber isolate (JN054635), India-eggplant isolates (GU906293, HQ343232), India-pepper isolates (KM272275, KM272276) and India-banana isolate (EF178298). It shared 94% nucleotide identity with Italy-pepper isolate (HE962480), India-chrysanthemum isolate (EF153733) and India-bottle gourd isolate (KJ874250). It showed 92% homology with Iran-squash isolate (JX025989), Iran-tomato isolate (JX025999) and Bangladesh-eggplant isolates (KM516898, KM516899) while showed 90% homology with South Korea-pepper isolate (KC527749) and tomato isolates of India (GU111229, JF279606) and Spain (AM183116, AJ829779). It showed 89% homology with tomato isolates of Spain (AJ829778) and China (EF216867).

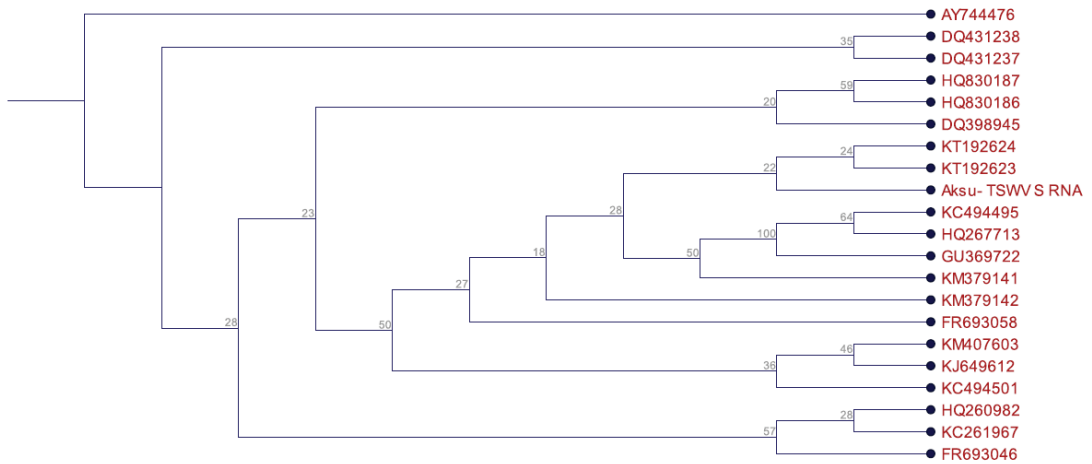

Figure 4- Phylogenetic tree analysis of Aksu isolate based on S RNA segment of TSWV

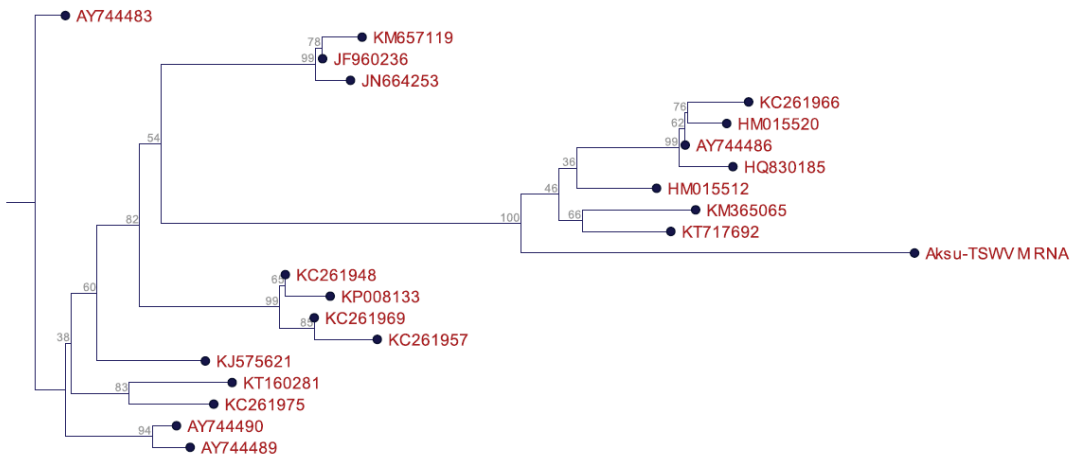

Figure 5- Phylogenetic tree analysis of Aksu isolate based on M RNA segment of TSWV

Table 4- Features of world isolates compared to Demre-CMV isolate

Isolates compared to Demre-CMV RNA 1		
GenBank Accession number	Origin	Host
KT921314	Egypt	Tomato
JN054636	Malaysia	Cucumber
KM272277	India	Pepper
HE962478	Italy	Pepper
KC527794	South Korea	Pepper
LC066420	Japan	Spinach
HF572914	USA	Bean
HE793683	France	Tomato
AM183117	Spain	Tomato
AB368499	Japan	Tomato
KM047509	South Korea	Potato
JN180309	South Korea	Corn
AB188231	Japan	Cucumber
EU159528	India	Banana
KC527785	South Korea	Pepper
KC527784	South Korea	Pepper
EF216866	China	Tomato
KC527787	South Korea	Pepper
EF213023	China	Chinese cabbage
KC527789	South Korea	Pepper
Isolates compared to Demre-CMV RNA 3		
GenBank Accession number	Origin	Host
GU906293	India	Eggplant
JN054635	Malaysia	Cucumber
KM272275	India	Pepper
HE962480	Italy	Pepper
KC527749	South Korea	Pepper
JX025989	Iran	Squash
JX025999	Iran	Tomato
KM516898	Bangladesh	Eggplant
AM183116	Spain	Tomato
EF153733	India	Chrysanthemum
HQ343232	India	Eggplant
EF178298	India	Banana
KJ874250	India	Bottle gourd
AJ829779	Spain	Tomato
EF216867	China	Tomato
JF279606	India	Tomato
KM272276	India	Pepper
KM516899	Bangladesh	Eggplant
AJ829778	Spain	Tomato
GU111229	India	Tomato

Table 5- Features of world isolates compared to Aksu-TSWV isolate

Isolates compared to Aksu-TSWV S RNA		
GenBank Accession number	Origin	Host
KT192623	Turkey, Samsun	Tomato
KM379141	Turkey, Samsun	Pepper
(*Tsw* resistance breaking isolate)		
KM407603	Turkey, Antalya	Pepper
KC494501	New Zealand	Tomato
FR693058	France	Tomato
FR693046	France	Pepper
DQ431237	Italy	Pepper
(*Tsw* resistance breaking isolate)		
DQ431238	Italy	Pepper
KM379142	Turkey, Samsun	Pepper
(Non-resistance breaking isolate)		
KJ649612	Hungary	Pepper
KT192624	Turkey, Samsun	Tomato
AY744476	USA	Dahlia
KC494495	New Zealand	Chrysanthemum
HQ830187	Italy	Pepper
HQ830187	Italy	Pepper
DQ398945	Italy	Pepper
(Resistance breaking isolate)		
KC261967	South Korea	Lettuce
HQ267713	South Korea	Pepper
HQ260982	South Korea	Pepper
GU369722	Italy	Pepper
Isolates compared to Aksu-TSWV M RNA		
GenBank Accession number	Origin	Host
AY744486	USA	Dahlia
HM015520	Spain	Tomato
HM015512	Spain	Tomato
KC261966	South Korea	Lettuce
HQ830185	Italy	Pepper
(Resistance breaking isolate)		
KT717692	Australia	Pepper
KM365065	Australia	Tomato
KP008133	Spain	Pepper
(*Tsw* resistance breaking isolate)		
AY744489	USA	Pepper
KC261957	South Korea	Pepper
KJ575621	Italy	Pepper
KC261969	South Korea	Tomato
JN664253	China	Lettuce
AY744483	USA	Chrysanthemum
KM657119	China	Pepper
KC261975	South Korea	Chrysanthemum
KC261948	South Korea	Pepper
KT160281	USA	Pepper
JF960236	China	Tomato
AY744490	USA	Tobacco
Isolates compared to Aksu-TSWV L RNA		
GenBank Accession number	Origin	Host
KP008130	Spain	Tomato
KC494520	New Zealand	Tomato
KJ575619	Italy	Pepper
KP008128	Spain	Tomato
KC261965	South Korea	Lettuce
KT717691	Australia	Pepper
KM365064	Australia	Tomato
KC261956	South Korea	Pepper
KP008132	Spain	Pepper
(*Tsw* resistance breaking isolate)		
KT160280	USA	Pepper
HM581934	South Korea	Tomato
KM657122	China	Pepper
KM657121	China	Tobacco
JN664254	China	Lettuce
JF960237	China	Tomato
KC261974	South Korea	Chrysanthemum
KC494508	New Zealand	Pepper
KC261947	South Korea	Pepper
KC261968	South Korea	Tomato
HM581937	South Korea	Pepper

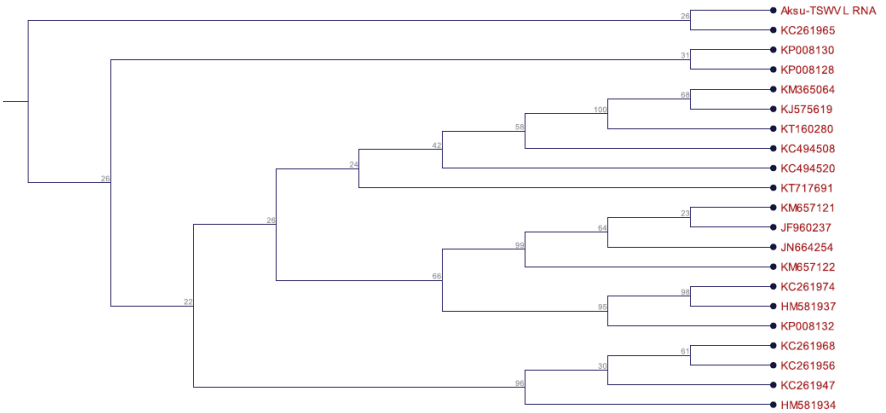

Figure 6- Phylogenetic tree analysis of Aksu isolate based on L RNA segment of TSWV

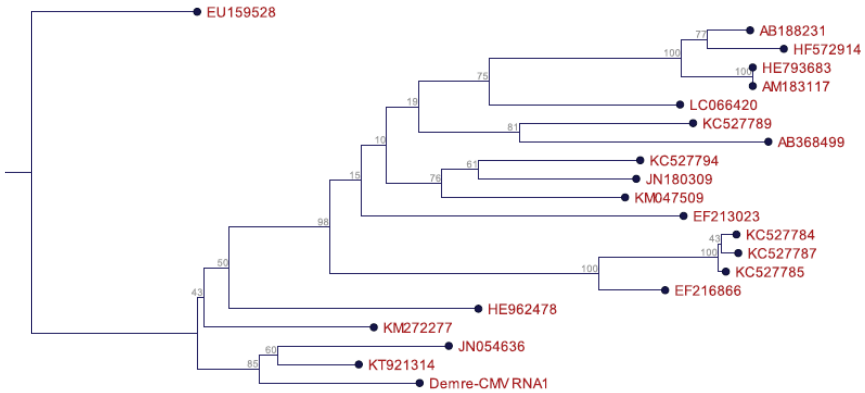

Figure 7- Phylogenetic tree analysis of Demre isolate based on RNA 1 segment of CMV

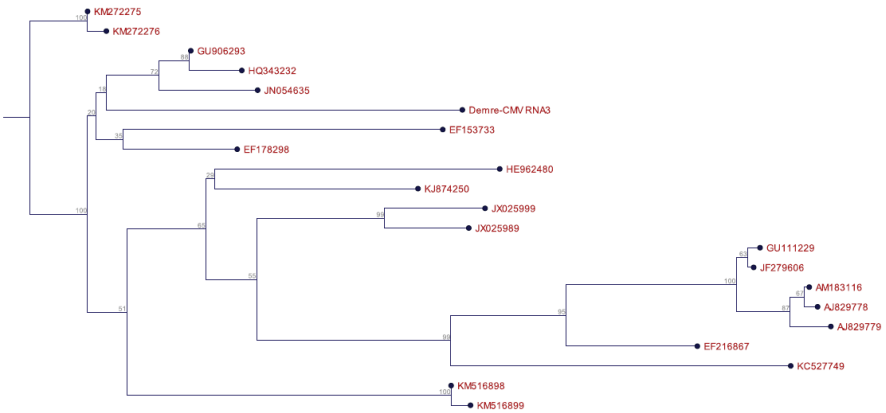

Figure 8- Phylogenetic tree analysis of Demre isolate based on RNA 3 segment of CMV

4. Discussion

The findings of the present study based on serological and molecular detection demonstrated the occurrence of TSWV and CMV causing damage in pepper plants in some districts of Antalya province. In our study, foliar symptoms of infected pepper plants were mosaic, mottling, chlorotic spots, necrotic spots, ring spots, chlorosis, curling and leaf deformation. Fruits were showing necrotic spots, chlorotic ring spots and roughness. Also, infected plants were showing curly top and stunting symptoms. TSWV can cause infection in pepper production areas worldwide and the rate is up to 100%. Infections are generally tested in Turkey and around the world using electron microscopy, ELISA and RT-PCR methods (Mavric & Ravnikar 2001; Sharman & Persley 2006; Ferrand et al 2015). TSWV infection has been detected 67.16% of pepper plants in the Western Mediterranean region of Turkey (Yardımcı & Çulal Kılıç 2009). CMV infection in pepper plants have been identified in many countries using methods such as electron microscopy, biological indexing, DAS-ELISA, RT-PCR and infection rates have been found to vary within the years (Burgmans et al 1986; Vozelj et al 2003; Biswas et al 2013). The studies were conducted in Turkey by using methods such as biological indexing, RT-PCR and RFLP but DAS-ELISA method was mostly used one and the infection in rates were determined in some provinces. In the samples collected from the pepper production areas in Samsun, DAS-ELISA test was used to detect CMV infection. Incidence of CMV was found 7.7% of 222 samples collected in 1998 while 2% of 91 samples were collected in 1999 (Arli-Sokmen et al 2005). In the pepper production areas of Hatay, Şanlıurfa, Kahramanmaraş and Gaziantep provinces 8.3% of CMV infection were detected by DAS-ELISA method (Buzkan et al 2006). In the pepper samples collected from Bursa, Yalova, İstanbul, Bilecik and Sakarya provinces 69% of CMV infection were detected using DAS-ELISA and real-time RT-PCR methods (Uzunoğulları & Gümüş 2015). CMV infected plants were showing mosaic, leaf deformation and stunting in our study. Disease incidence in the surveyed samples from different districts from Antalya, TSWV was found major virus as compared to CMV among the DAS-ELISA tested pepper samples. Further effort was also made to partial molecular characterization of TSWV and CMV from infected plants. The sequence obtained by amplification of the region of the S RNA segment of Aksu isolate showed high homology with *Tsw* resistance breaking pepper isolate from Samsun and Italy. The sequence of the region from the M RNA segment of Aksu isolate showed homology with *Tsw* resistance breaking pepper isolate from Italy and Spain. The sequence of the region of the L RNA segment of Aksu isolate showed homology with *Tsw*-resistance breaking pepper isolate from Spain. There are several reports displaying that TSWV resistance breaking isolates have emerged in Italy (Roggero et al 2002), Spain (Margaria et al 2004) and Australia (Sharman & Persley 2006). Also, TSWV resistance-breaking strains have been reported from Hungary (Gabor et al 2012), Argentina (Ferrand et al 2015) and Turkey (Deligoz et al 2014) in Capsicum species carrying the *Tsw* gene. In several reports it has been identified that the genetic determinant for overcoming pepper *Tsw* resistance is located in S segment but amino acid substitutions responsible for TSWV breakdown remain unidentified (Debreczeni et al 2015). The sequence obtained by amplification of the region of the RNA 1 segment of Demre isolate showed high homology with Egypt-tomato isolate and Malaysia-cucumber isolate. The sequence of the region of the RNA 3 segment of Demre isolate showed high homology with Malaysia-cucumber isolate, India-eggplant isolate and India-pepper isolate. There is a report that CMV resistance breaking isolate has been described in Korea. Sequence homology of RNA 3 segment of resistance breaking CMV isolate revealed high similarity with known CMV strains. The resistance and resistance-breaking mechanisms of CMV in pepper remain to be investigated (Lee et al 2006).

5. Conclusions

Virus resistant pepper cultivars are important management technique for TSWV and CMV control in pepper. These resistances are mostly based on the resistance genes and they have broken down. There is a need to understand the ability of TSWV and CMV isolates to overcome resistance which can be further useful in breeding programs to develop pepper cultivars resistance against TSWV and CMV. Further effort should be taken to identify TSWV and CMV breakdown mechanism of resistance breaking TSWV and CMV isolates.

References

Adkins S (2000). Tomato spotted wilt virus-positive steps towards negative success, *Molecular Plant Pathology* 1(3): 151-157

Arli-Sokmen M A, Mennan H, Sevik M A & Ecevit O (2005). Occurrence of viruses in field-grown pepper crops and some of their reservoir weed hosts in Samsun, Turkey, *Phytoparasitica* 33(4): 347-358

Biswas K, Hallan V, Zaidi A A & Pandey P K (2013). Molecular evidence of Cucumber mosaic virus subgroup II infecting Capsicum annuum L. in the Western region of India, *Current Discovery* 2(2): 97-105

Burgmans J L, Fry P R & Sunde R G (1986). Peppers: survey of virus diseases of Capsicum annuum in Hawke's Bay and Poverty Bay, *New Zealand Journal of Experimental Agriculture* 14: 459-463

Buzkan N, Demir M, Öztekin V, Mart C, Çağlar B K & Yilmaz M A (2006). Evaluation of the status of capsicum viruses in the main growing regions of Turkey, *EPPO Bulletin* 36: 15-19

De Blas C, Borza M J, Saiz M & Romero J (1994). Broad spectrum detection of Cucumber mosaic virus (CMV) using the polymerase chain reaction, *Journal of Phytopathology* 141: 323-329

Debreczeni D E, López C, Aramburu J, Darós J A, Soler S, Galipienso L, Falk R B & Rubio L (2015). Complete sequence of three different biotypes of Tomato spotted wilt virus (wild type, tomato Sw-5 resistance-breaking and pepper Tsw resistance-breaking) from Spain, *Archives of Virology* 160(8): 2117-2123

Deligoz I, Sokmen M A & Sari S (2014). First report of resistance breaking strain of Tomato spotted wilt virus (Tospovirus; Bunyaviridae) on resistant sweet pepper cultivars in Turkey, *New Disease Reports,* 30: 26

Ferrand L, García M L, Resende R O, Balatti P A & Dal Bó E (2015). First report of a resistance-breaking isolate of Tomato spotted wilt virus infecting sweet pepper harboring the Tsw gene in Argentina, *Plant Disease* 99(12): 1869-1870

Foissac X, Svanella-Dumas L, Dulucq M J, Candresse T & Gentit P (2001). Polyvalent detection of fruit tree tricho, capillo and foveaviruses by nested RT-PCR using degenerated and inosine containing primers (PDO RT-PCR), *Acta Hortic* 550: 37-43

Gabor B, Krizbai L, Horvath J & Takacs A (2012). Resistance breaking strain of Tomato spotted wilt virus (TSWV) on resistant pepper cultivars in Hungary, *Proceedings of the International Symposium on Current Trends in Plant Protection,* 25-28th September, Belgrade, Serbia, pp. 239-241

Kumari R, Bhardwaj P, Singh L, Zaidi A A & Hallan V (2013). Biological and molecular characterization of Cucumber mosaic virus subgroup II isolate causing severe mosaic in cucumber, *Indian Journal of Virology* 24(1): 27-34

Lee M Y, Lee J H, Ahn H I, Yoon J Y, Her N H, Choi J K, Choi G S, Kim D S, Harn C H & Ryu K H (2006). Identification and sequence analysis of RNA3 of a resistance-breaking Cucumber mosaic virus isolate on Capsicum annuum, *The Plant Pathology Journal* 22(3): 265-270

Margaria P, Ciuffo M & Turina M (2004). Resistance breaking strain of Tomato spotted wilt virus (Tospovirus; Bunyaviridae) on resistant pepper cultivars in Almeria Spain, *Plant Pathology* 53(6): 795-795

Mavric I & Ravnikar M (2001). First report of Tomato spotted wilt virus and Impatiens necrotic spot virus in Slovenia, *Plant Disease* 85(12): 1288

Mound L A (2001). So many thrips-so few tospoviruses, *Thrips and Tospoviruses: Proceedings of the VII. International Symposium on Thysanoptera,* 1–8 July, Reggio Calabria, Italy, pp. 15-18

Moury B & Verdin E (2012). Viruses of pepper crops in the Mediterranean basin: a remarkable stasis, *Advances in Virus Research* 84: 127-162

Nour S M, Maleki M & Ghotbi T (2013). Biological and serological detection of TSWV on three commercial cultivars Chrysanthemum morifolium in Markazi

province of Iran, *Annals of Biological Research* 4(4): 112-119

Perry K L, Zhang L & Palukaitis P (1998). Amino acid changes in the coat protein of Cucumber mosaic virus differentially affect transmission by the aphids Myzus persicae and Aphis gossypii, *Virology* 242: 204-210

Roggero P, Masenga V & Tavella L (2002). Field isolates of Tomato spotted wilt virus overcoming resistance in pepper and their spread to other hosts in Italy, *Plant Disease* 86: 950-954

Sharman M & Persley D M (2006). Field isolates of Tomato spotted wilt virus overcoming resistance in Capsicum in Australia, *Australasian Plant Pathology* 35: 123-128

Uzunoğulları N & Gümüş M (2015). Marmara bölgesi'nde bazı kültür bitkilerinde doğal enfeksiyona neden olan hıyar mozaik virüsü (Cucumber mosaic virus, CMV)'nün tespiti, *Trakya University Journal of Natural Sciences* 16(1): 9-15

Vozelj N, Petrovic N, Novak M P, Tusek M, Mavric I & Ravnikar M (2003). The most frequent viruses on selected ornamental plants and vegetables in Slovenia, *Zbornik predavanj in referatov 6. Slovenskega Posvetovanje o Varstvu Rastlin*, Zrece pp. 300-304

Yardımcı N & Çulal Kılıç H (2009). Tomato spotted wilt virus in vegetable growing areas in the West Mediterranean region of Turkey, *African Journal of Biotechnology* 8(18): 4539-4541

Yılmaz M A & Davis R F (1985). Identification of viruses infecting vegetable crops along the Mediterranean Sea coast in Turkey, *The Journal of Turkish Phytopathology* 14: 1-8