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Current Status of Some Cucurbit Viruses in Cukurova Region (Adana and Mersin Provinces) of Turkey and Molecular Characterization of *Zucchini Yellow Mosaic Virus* Isolates

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Abstract

Surveys were conducted from 2008 to 2010 to identify the viruses infecting cucurbit fields in Adana and Mersin provinces of Cukurova region in Turkey. All cucurbit samples collected from 18 districts were tested for Cucumber mosaic virus (CMV), Cucurbit aphid-borne yellow virus (CABYV), Papaya ringspot virus (PRSV), Squash mosaic virus (SqMV), Zucchini yellow mosaic virus (ZYMV) and Watermelon mosaic virus (WMV) by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using polyclonal antisera. Overall, 449 out of 485 samples were found positive for the tested viruses; 327 out of 350 samples in Adana and 122 out of 135 in Mersin were infected at least with one virus. During the entire surveys, 258 of 281 squash samples, 33 of 33 cucumber samples, 84 of 88 melon samples and 74 of 83 watermelon samples were found to have a single or mix virus infection. All six viruses were detected on cucurbits in Adana while all samples from Mersin province were found to be negative for CABY.

Sequence analyses indicated that the Turkish ZYMV isolates (ZYMV-TR3, ZYMV-TR15 and ZYMV-TR17) belong to the most common molecular cluster of ZYMV worldwide, including isolates from Europe, Africa, the Middle East, the Indian subcontinent and South America.

Keywords: *Cucurbits, ELISA, RT-PCR, ZYMV, Molecular characterization*

1. Introduction

Cucurbits are among the most important vegetable crops and they represent 50% of edible vegetable production in Adana and Mersin provinces in Cukurova region of Turkey. According to the reports of the Turkish Statistical Institute in 2012, total production of cucurbits in Cukurova region was 1,37 million tons (approximately 18% of cucurbit production of Turkey) covering an area of 22,870 ha. Among cucurbits watermelon occupied top position with 943,422 tons followed by cucumber, melon and squash with 208,902 tons, 141,296 tons and 69,603 tons, respectively, in Cukurova region [1].

Viral diseases significantly affect both, the yield and quality of these crops and cause severe economic losses. More than sixty viruses infect and this number is increasing with new viral agents every year [2]. *Cucumber mosaic virus* (CMV), *Cucurbit aphid-borne yellow virus* (CABYV), *Papaya ringspot virus* (PRSV), *Squash mosaic virus* (SqMV), *Zucchini yellow mosaic virus* (ZYMV) and *Watermelon mosaic virus* (WMV) are widely spread viruses on cucurbits in the Mediterranean region. Whereas CMV, PRSV, ZYMV and WMV are transmitted by aphids in a non-persistent manner and CABYV is persistently aphid-transmitted, SqMV is transmitted primarily by beetles, contact and also by seeds in melon and squash. These viruses mostly cause systemic mosaic, mottle, vein banding, yellowing and deformation on the leaves and misshaping in fruits of infected cucurbits; CABYV induces

only leaf yellowing with less impact on fruit quality. The symptoms of infection can vary depending on the host species, the cultivar, environmental conditions and virus strains [2]. Some cucurbit viruses have previously been detected in studies conducted in different parts of Turkey. The presence of CMV, ZYMV and WMV has been reported in the Black Sea region (Northern Turkey) [3], CMV and ZYMV in Gaziantep province (Southeastern Turkey) [4], CMV, ZYMV, PRSV, SqMV and WMV in Thrace region (Northwest Turkey) [5], CMV, ZYMV and WMV in Samsun (Northern Turkey) [6] and ZYMV and CMV in Hatay province (Southeast Turkey) [7]. In recent years, ZYMV, CMV, WMV, PRSV, SqMV and CGMMV (*Cucumber green mottle mosaic Tobamovirus*) were detected in Ankara and ZYMV, CMV and WMV in Antalya [8]. In another study, ZYMV, CMV, WMV, PRSV and SqMV were reported in Konya province [9]. CMV infection has also been recently reported in parsley, water mint and globe artichoke in Turkey ([10, 11, 12]. There is no antiviral chemical substance available for controlling plant viruses. Therefore, detection and identification of the viruses and data collection on their prevalence and distribution in the fields are the most important steps in management of viral diseases. Up to now, there is no information available on the most prevalent cucurbit viruses and their frequency and distribution in the fields of growers in Cukurova region (Adana and Mersin provinces) in Turkey. This study was carried out to determine the prevalence of some economically important cucurbit-infecting viruses (CMV, CABYV, PRSV, SqMV, ZYMV and WMV) and investigate their occurence and incidence in Cukurova region between 2008 and 2010. In addition, partial capsid protein sequences of three selected ZYMV isolates were obtained and their genetic variability and phylogenetic relationship with worldwide ZYMV isolates were compared.

2. Materials and Methods

2.1. Surveys and sample collection

Surveys were conducted in 48 fields in Cukurova region from every June to October between 2008 and 2010. A total of 485 symptomatic plants (350 from Adana and 135 from Mersin) were sampled from cucumber, melon, squash and watermelon fields in Adana (City center, Gulbahcesi, Dogankent, Sirkenli, Karatas, Misis, Kucukdikili, Buyukdikili, Gunyurdu, Camilimanda, Karaisali, Salbas) and Mersin (City center, Gokceler, Yesiltepe, Kazanli, Yolgecen, Tarsus-Yenice) provinces of Turkey (Figure 1). The plants were observed for virus-type of symptoms such as mosaic, mottle, yellowing and deformation on the leaves and fruits. The samples from plants showing virus-like symptoms were placed in a plastic bag on ice and transported to the laboratory at the University of Cukurova. They were processed within 48 h of collection or kept at -20 °C until being tested.

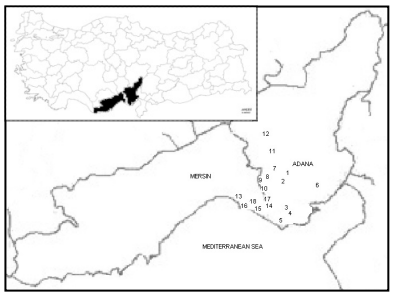

Figure 1. Map of districts in which surveys were conducted in Adana and Mersin provinces (Cukurova region). Map in the corner indicates the location of Adana and Mersin provinces in Turkey. Adana Province: 1. City center, 2. Gulbahcesi, 3. Dogankent, 4. Sirkenli, 5. Karatas, 6. Misis, 7. Kucukdikili, 8. Buyukdikili, 9. Gunyurdu, 10. Camilimanda, 11. Karaisali, 12. Salbas; Mersin province: 13. City center, 14. Gokceler, 15. Yesiltepe, 16. Kazanli, 17. Yolgecen, 18. Tarsus-Yenice

2.2. Serological detection

The presence of CMV, CABYV, PRSV, SqMV, ZYMV and WMV was investigated in all samples by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) according to the antisera manufacturer's procedure (BIOREBA AG, Reinach, Switzerland; SEDIAG S.A.S., Longvich, France). The samples were tested in the Plant Virology Laboratory, Department of Plant Protection, Faculty of Agriculture at the University of Cukurova.

Plant extracts from fresh leaves of the collected samples were added to ELISA plates coated with specific antisera and incubated at 4ºC overnight. After washing, enzyme conjugate was added and the plates were incubated at 30ºC for 4h. After one more washing step, substrate was added to the wells and the plates were incubated at room temperature for 60-120 min. Absorbance values were measured at 405 nm using an ELISA reader (MEDISPEC ESR-200). Virus free cucurbit plants, grown in an insect-proff greenhose, were used as negative control and samples with absorbance value two to three times higher than the negative control were considered to be infected [13]. All samples were tested in duplicate and their average results were taken.

2.3. Molecular characterization of ZYMV isolates

Three ZYMV positive samples were selected and used in molecular studies to characterize the genetic diversity among isolates of ZYMV (ZYMV-TR3, ZYMV-TR15 and ZYMV-TR17) found in Adana and Mersin provinces. Isolate ZYMV-TR3 was obtained from squash in Mersin province. Isolates ZYMV-TR15 and ZYMV-TR17 were obtained from squash and melon, respectively, in Adana province.

2.4. Total RNA extraction

Total RNA was extracted from ZYMV infected leaves according to Astruc *et al.* (1996) [14]. One gram tissue was extracted in TE buffer (100 mM Tris-HCl pH 8.0, 50 mM EDTA, 500 mM sodium chloride and 0.1% 2-mercapthoethanol) and centrifuged at 4 000 rpm for 5 min. Then 50 µl of 20% SDS were added into each tube and the tubes were kept at 65ºC for 15 min. After adding 250 µl of 6 M potassium acetate (pH 6.5), the tubes were transferred on ice for 20 min. Then nucleic acids were precipitated with 500 µl ethanol and the pellet was resuspended in 50 µl RNAse-free sterile water.

2.5. RT-PCR assays

RT- PCR reactions were performed in a thermal cycler (Techne TC 4000, Cambridge, UK) using ZYMV specific primers ZYMV-CP-5' (5'- GGTTCATGTCCCACCAAGC -3') and ZYMV-CP-3' (5'- ATGTCGAGTATCACATTTCC -3') [15], which yielded a 605 nucleotide (nt) fragment overlapping the N-terminal part of the coat protein (CP) and C terminal part of the polymerase coding regions (NIb).

Complementary DNA synthesis was done by using 2 µl of total RNA in reverse transcription in a final volume of 25 µl for 1h at 42ºC with M-MuLV reverse transcriptase (Fermentas, Vilnius, Lethuania). PCR was performed in 25 µ1 of mix containing 16.8 µl of sterile distilled water, 2.5 µl of *Taq* DNA polymerase buffer, 1.5 µl $MgCl_2$, 1 µl of dNTPs (10 mM), 1µl of each primer (10 mM), 1 unit of *Taq* polymerase and 2 µl of the cDNA. PCR cycling conditions included an initial denaturation of 2 min at 94ºC, 35 cycles of 30 sec at 94 º C, 30 sec at 55 º C and 2 min at 72 º C, and a final extension for 10 min at 72ºC. PCR products were analyzed by on a 1.5% agarose gel and viewed with a UV transilluminator after staining with ethidum bromide.

2.6. Nucleic acid sequencing and sequence analysis

The amplified PCR fragments were purified according to manufacturer's instructions with the Roche High Pure PCR Product Purification Kit. Sequencing was done by using automated DNA sequencer (Aplied Biosystems ABI 310) at Iontek Research and Biotechnology Company (Turkey).

The nucleotide sequences were compared with GenBank isolates using BLASTn [16]. Sequences were aligned with *ClustalW* included in MEGA5 [17]. A neighbour-joining tree was reconstructed with 500 bootstrap replicates using MEGA5.

3. Results

3.1. Frequency of cucurbit viruses

During the three-years surveys, a total of 485 samples were collected in Cukurova region (Adana and Mersin provinces) and 449 samples were found to be positive (92.6%) for the investigated viruses. ZYMV was the most prevalent virus detected in 203 samples (45.2%) followed by WMV in 65 samples (14.5%). ZYMV+WMV, ZYMV+CMV and ZYMV+CMV+WMV were the most common mixed infection types. However, mixed infection was not detected in cucumber in Adana province and in cucumber and melon samples in Mersin province. ZYMV was the most frequently virus found in cucumber, squash and melon with infection rates of 51.5%, 46.1% and 44.0%, respectively, while ZYMV and WMV were detected in 40.5% of the watermelon samples in Cukurova region (Table 1).

Table 1. Types and number of virus infection in cucurbit samples in Cukurova Region

	Number of virus infected samples				
Types of Infection	**Squash**	**Cucumber**	**Melon**	**Watermelon**	**Total**
ZYMV	119	17	37	30	**203**
CMV	13	9	5	1	28
WMV	16	5	14	30	**65**
PRSV	10	-	-	2	12
SqMV	5	2	13	-	20
CABYV	2	-	-	-	2
ZYMV+CMV	7	-	7	-	14
ZYMV+WMV	30	-	5	1	**36**
ZYMV+PRSV	9	-	-	-	9
ZYMV+SqMV	7	-	3	-	10
CMV+WMV	9	-	-	3	12
ZYMV+ CABYV	2	-	-	-	2
SqMV+PRSV	3	-	-	-	3
SqMV+WMV	3	-	-	-	3
WMV+CABYV	6	-	-	-	6
ZYMV+PRSV+SqMV	1	-	-	-	1
ZYMV+PRSV+WMV	1	-	-	1	2
ZYMV+SqMV+WMV	1	-	-	2	3
ZYMV+CMV+WMV	13	-	-	4	**17**
ZYMV+CMV+SqMV+WMV	1	-	-	-	1
Positive	**258**	**33**	**84**	**74**	**449**
Negative	**23**	**-**	**4**	**9**	**36**
Tested (Total)	**281**	**33**	**88**	**83**	**485**

Considering the provinces and plant species; out of the 350 cucurbit samples tested, 327 were infected in Adana province (93.4%). Among the samples, 230 were found to be infected with only one virus (65.7%) while 97 samples were infected with more than one virus (27.7%). ZYMV was the most common virus (42.8% of the samples) followed by WMV (13.5%). ZYMV, CMV and WMV were detected in all species of the tested cucurbit samples. However, CABYV was assigned only in two squash samples. ZYMV was found to be the most prevalent virus in squash and melon, WMV was the most common virus in watermelon and ZYMV and CMV in cucumber (Table 2).

Table 2. Types and number of virus infection in cucurbit samples in Adana Province

	Number of virus infected samples				
Infection Types	**Squash**	**Cucumber**	**Melon**	**Watermelon**	**Total**
ZYMV	90	7	32	11	140
CMV	8	7	5	1	21
WMV	9	5	14	16	44
PRSV	8	-	-	2	10
SqMV	5	2	6	-	13
CABYV	2	-	-	-	2
ZYMV+CMV	3	-	7	-	10
ZYMV+WMV	25	-	5	1	31
ZYMV+PRSV	8	-	-	-	8
ZYMV+SqMV	6	-	3	-	9
CMV+WMV	6	-	-	3	9
ZYMV+ CABYV	2	-	-	-	2
SqMV+PRSV	3	-	-	-	3
SqMV+WMV	3	-	-	-	3
ZYMV+PRSV+SqMV	1	-	-	-	1
ZYMV+PRSV+WMV	1	-	-	1	2
ZYMV+SqMV+WMV	1	-	-	2	3
ZYMV+CMV+WMV	13	-	-	2	15
ZYMV+CMV+SqMV+WMV	1	-	-	-	1
Positive	**195**	**21**	**72**	**39**	**327**
Negative	**13**	**-**	**2**	**8**	**23**
Total	**208**	**21**	**74**	**47**	**350**

Cucurbits collected from all districts of Adana province were found be infected with ZYMV and the highest rate of ZYMV infection was detected in Kucuk Dikili district. WMV was the most prevalent virus in Camilimanda district. CABYV was not common and detected only in Karaisalı and Salbas (Table 3).

In Mersin province, 135 cucurbit samples were tested and 122 were found to be infected with the investigated viruses (90.4%). Out of the 135 samples, 100 were infected with one virus (74.0%) and 22 samples had multiple infections (16.3%). Thirteen samples were negative. ZYMV was the most prevalent virus (51.6%), followed by WMV (17.2%). CABYV was detected only in mixed infection with WMV. ZYMV was the most common virus in squash, cucumber, melon and watermelon, while SqMV was detected only in melon and PRSV in squash samples. In addition to these, double infection types were assigned only in squash samples (Table 4).

All tested viruses were detected in single or in mixed infection in Mersin province. The highest infection rate of ZYMV, the most prevalent virus in the region, was obtained in Center (87.5%) followed by Yolgecen (75%) and Tarsus-Yenice (61.1%) districts. PRSV and SqMV were detected only in squash and melon samples, respectively, in Kazanli (Table 5).

Table 3. Distribution of infection types of the tested viruses in the districts of Adana province

Infection Types	Center	Gulbahcesi	Dogankent	Sirkenli	Karatas	Misis	Kucuk Dikili	Buyuk Dikili	Gunyurdu	Camilimanda	Karaisali	Salbas	Total
ZYMV	16	1	18	26	3	14	29	9	6	5	2	11	140
CMV	3	6	5	-	-	6	1	-	-	-	-	-	21
WMV	-	5	9	2	-	4	2	-	-	19	3	-	44
PRSV	3	-	7	-	-	-	-	-	-	-	-	-	10
SqMV	5	-	-	-	2	-	3	-	3	-	-	-	13
CABYV	-	-	-	-	-	-	-	-	-	-	1	1	2
ZYMV+CMV	-	1	2	-	6	-	-	-	-	1	-	-	10
ZYMV+WMV	2	-	7	-	1	6	5	-	-	5	5	-	31
ZYMV+PRSV	3	-	2	-	-	-	3	-	-	-	-	-	8
ZYMV+SqMV	2	-	2	-	-	-	2	-	3	-	-	-	9
CMV+WMV	-	5	1	-	-	-	-	-	-	3	-	-	9
ZYMV+CABYV	-	-	-	-	-	-	-	-	-	-	2	-	2
SqMV+PRSV	3	-	-	-	-	-	-	-	-	-	-	-	3
SqMV+WMV	2	-	-	-	-	-	1	-	-	-	-	-	3
ZYMV+PRSV+SqMV	-	-	-	-	-	-	1	-	-	-	-	-	1
ZYMV+PRSV+WMV	1	-	1	-	-	-	-	-	-	-	-	-	2
ZYMV+SqMV+WMV	-	-	2	-	-	-	1	-	-	-	-	-	3
ZYMV+CMV+WMV	-	-	-	-	2	6	-	-	-	7	-	-	15
ZYMV+CMV+SqMV+WMV	-	-	-	-	-	-	1	-	-	-	-	-	1
Positive	**40**	**18**	**56**	**28**	**14**	**36**	**49**	**9**	**12**	**40**	**13**	**12**	**327**
Negative	**3**	**-**	**5**	**2**	**-**	**3**	**-**	**4**	**-**	**4**	**-**	**2**	**23**
Total	**43**	**18**	**61**	**30**	**14**	**39**	**49**	**13**	**12**	**44**	**13**	**14**	**350**

Table 4. Types and number of virus infection in cucurbit samples in Mersin province

	Number of virus infected samples				
Infection Types	**Squash**	**Cucumber**	**Melon**	**Watermelon**	**Total**
ZYMV	29	10	5	19	63
CMV	5	2	-	-	7
WMV	7	-	-	14	21
PRSV	2	-	-	-	2
SqMV	-	-	7	-	7
ZYMV+CMV	4	-	-	-	4
ZYMV+WMV	5	-	-	-	5
ZYMV+PRSV	1	-	-	-	1
ZYMV+SqMV	1	-	-	-	1
CMV+WMV	3	-	-	-	3
WMV+CABYV	6	-	-	-	6
ZYMV+CMV+WMV	-	-	-	2	2
Positive	**63**	**12**	**12**	**35**	**122**
Negative	**10**	**-**	**2**	**1**	**13**
Total	**73**	**12**	**14**	**36**	**135**

Table 5. Distribution of infection types of the tested viruses in the districts of Mersin province

Infection Types	Center	Tarsus-Yenice	Yesiltepe	Kazanli	Yolgecen	Gokceler	Total
ZYMV	7	22	-	19	12	3	63
CMV	1	4	-	2	-	-	7
WMV	-	-	4	7	4	6	21
PRSV	-	-	-	2	-	-	2
SqMV	-	-	-	7	-	-	7
ZYMV+CMV	-	3	-	1	-	-	4
ZYMV+WMV	-	3	-	1	-	1	5
ZYMV+PRSV	-	-	-	1	-	-	1
ZYMV+SqMV	-	-	-	1	-	-	1
CMV+WMV	-	2	-	1	-	-	3
WMV+CABYV	-	-	6	-	-	-	6
ZYMV+CMV+WMV	-	2		-	-	-	2
Positive	**8**	**36**	**10**	**42**	**16**	**10**	**122**
Negative	**10**	**-**	**1**	**-**	**2**	**-**	**13**
Total	**18**	**36**	**11**	**42**	**18**	**10**	**135**

3.2. Nucleic acid sequencing and sequence analysis

RT-PCR-amplified fragments of approximately 605 bp were obtained using primers ZYMV-CP-5'and ZYMV-CP-3' and sequenced. The sequences were deposited into Genbank under accessions numbers of KP871836- KP871838.

Sequence analyses showed high sequence conservation between Turkish isolates of ZYMV (98% identity), and up to 98% identity with isolates from different parts of the world. In the N-terminal part of the capside protein gene of Turkish isolates shared 95-98% identity with isolates "Ahlat" (Genbank accession JF317297) and "Adana" (JF317296) collected from Turkey in 2011.

A phylogenetic tree was constructed from the sequences of the CP genes of 43 selected ZYMV isolates together with the Turkish virus isolates, ZYMV-TR3, ZYMV-TR15 and ZYMV-TR17. As previously known, ZYMV isolates were grouped into three main clusters corresponding to the groups A, B and C [18] (Figure 2). Group A comprises six subclusters (A1-A6). The three Turkish isolates of ZYMV belonged to subcluster A1, the largest one, containing isolates from Europe (France, Germany, Hungary, Slovakia, Poland...), Africa (Sudan, Ivory Coast...), the Middle East (Iran, Israel, Syria...), South America (Venezuela, Brazil) and Asia (India, Pakistan).

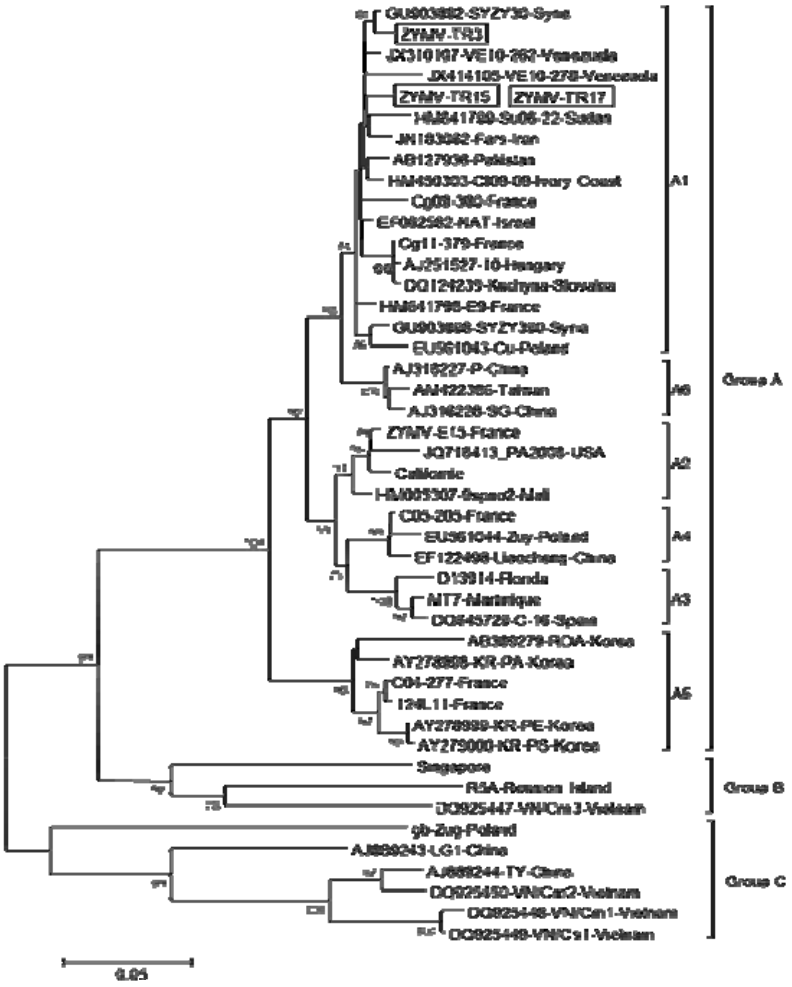

Figure 2. Neighbour-joining tree was constructed with nucleotides sequences from a 605-nt fragment of the CP gene of 3 isolates of ZYMV from Turkey and 43 isolates of ZYMV from different parts of the world. Bootstrap values (500 replicates) above 60% are indicated as percentage for each node

4. Discussion

A large scale survey was conducted for detection of some major viruses, CMV, CABYV, PRSV, SqMV, WMV and ZYMV in squash, cucumber, melon and watermelon fields in Adana and Mersin provinces (Cukurova region) by using ELISA method.

All of the six viruses were detected in the collected samples. Most of the samples collected in this study were found to be infected with at least one of the investigated viruses (92.6%). However, thirty six samples (7.4%) did not react positively with any of the antisera used in ELISA, even though virus-like symptoms such as mosaic, mottling, yellowing, dwarfing and deformation on the leaves and blistering on the fruits were observed in the plants. Abiotic factors including unsuitable environmental conditions, mineral deficiencies such as nitrogen, zinc or iron, phytotoxicity of pesticide or nutrients or possible infection of some other viruses not tested in the current study could explain these symptoms. In addition to these, the status of whitefly-transmitted viruses on cucurbits in Turkey is not well-known, although the crinivirus *Cucurbit yellow stunting disorder vius* (CYSDV) has been present in Turkey since at least 1996 [19] and the ipomovirus *Cucumber vein yellowing virus* (CVYV)

has been reported recently [20]. However, some other viruses present in neighbouring countries are: the begomoviruses *Watermelon chlorotic stunt virus* (WmCSV) and *Tomato leaf curl Palampur virus* (TLCPaV) in Iran [21, 22], as well as CVYV and the criniviruses CYSDV and *Cucumber chlorotic yellows virus* (CCYV) [23, 24]; CVYV, CYSDV and the crinivirus *Beet pseudo-yellows virus* (BPVY) in Cyprus [25]; CCYV and the aphid-transmitted potyvirus *Moroccan watermelon mosaic virus* (MWMV in Greece [26, 27].

Our results indicated that ZYMV was the most widespread virus followed by WMV, CMV, SqMV, PRSV and CABYV in Cukurova region. In the studies made on cucurbit viruses in Turkey and different countries of the world, various results were obtained. While ZYMV was the most widespread virus in watermelon and melon in Thrace region [5], WMV infection was found to be the most common (53.9%) in Samsun province, and ZMYV and CMV were in Gaziantep province of Turkey with the rates of 40% and 36%, respectively [6, 4]. Although CABYV was found very low in Turkey, it was reported as one of the most prevalent virus of field-grown cucurbits in Lebanon and in the different regions of Iran along with ZYMV [28, 29]. The low prevalence of CABYV in Turkey is rather unexpected, since the virus appears frequent also in Cyprus [30] and several European countries [31, 2]. Differences in local aphid or weed reservoir populations may account for this situation in Turkey.

The percentages of samples infected with at least one of the six viruses were 69.2-100% in the different districts of Adana and 44.4-100% in the districts of Mersin. Except SqMV and CABYV, all viruses detected in this study were non-persistently aphid-borne by many different aphid species. ZYMV was found to be the most abundant virus in most of the districts. The high frequency of ZYMV suggests its local maintenance throughout the whole year in reservoir plants near the crops, allowing frequent and early epidemics, contrary to more northern countries including France where reservoirs appear scarce and ZYMV epidemics are very irregular [32].

In the second part of this work, the molecular variability of three Turkish isolates of ZYMV from Adana and Mersin provinces was studied. Molecular analysis of the N-terminal part of the coat protein (CP) and C terminal part of the polymerase coding regions (NIb) indicated very low genetic diversity among Turkish isolates. Geographically, Adana and Mersin regions are close to each other the source of the isolates may be the same. Turkish isolates clustered in the same group with isolates from different parts of world such as Europe, Africa, the Middle East and South America. In several cases, it has been shown that there was no obvious relation between isolates and their geographic distribution. The international trade of infected fruit, plants, or seeds can be explanations of the widespread geographic distribution of similar ZYMV isolates [33], and on the opposite the lack of relation sometimes observed between isolates from neighboring countries [34, 35]. It was confirmed that seed transmission (vertical transmission) takes place in ZYMV and infected seeds may contribute to virus maintenance and long-distance dispersion, however the real agronomic impact of ZYMV seed transmission remains to be determined [36].

As reported previously that because of the high incidence of aphid transmitted viruses, the use of mulches, floating covers, vector targeting insecticides and resistant cucurbit cultivars could be suggested to the growers to control the spread and distribution of cucurbit viruses in the region [37]. In addition, further studies are needed to explore previously unreported viruses in cucurbits in Cukurova region and different locations of Turkey, and also epidemiology and characterization of Turkish isolates of cucurbit viruses in order to develop feasible control recommendations.

5. Conclusions

Some major cucurbit viruses (CMV, CABYV, PRSV, SqMV, ZYMV and WMV) were detected in single and mixed infections in Cukurova region (Adana and Mersin provinces) by ELISA. While ZYMV was found to be the most prevalent virus, CABYV was not common in the region. The results of sequence analysis showed that Turkish ZYMV isolates belong to molecular subcluster A1 in a group A which is the most ubiquitous group worldwide. The data obtained by this study may help better understand the frequency and distribution of some economically important cucurbit viruses and genetic variability of ZYMV isolates and phylogenetic relationship with worldwide ZYMV isolates in Turkey.

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