ردیابی و شناسایی مولکولی چهار ویروس مهم مزارع صیفی‌جات در غرب و شمال‌غرب ایران*

کژال محمدی[۱]، محمد حاجی‌زاده[۱]** و داود کولیوند[۲]

(تاریخ دریافت: ۱۳۹۴/۱۱/۳۰؛ تاریخ پذیرش: ۱۳۹۵/۳/۲۳)

چکیده

در این مطالعه، ردیابی و شناسایی ویروس‌های مهم میزبان‌های صیفی در غرب و شمال غرب ایران طی سال‌های ۱۳۹۲ و ۱۳۹۳ مورد بررسی قرار گرفت. به این منظور، تعداد ۱۰۰ نمونه برگی از میزبان‌های مختلف که مشکوک به آلودگی با ویروس‌های موزاییک خیار (*Cucumber mosaic virus*, CMV)، موزاییک پیسک سبز خیار (*Cucumber green mottle mosaic virus*, CGMMV)، موزاییک هندوانه (*Watermelon mosaic virus*, WMV) و موزاییک زرد کدو (*Zucchini yellow mosaic virus*, ZYMV) بودند، جمع‌آوری و آلودگی نمونه‌ها با روش RT-PCR و استفاده از آغازگرهای اختصاصی هر ویروس تعیین شد. نتایج نشان داد که بیشترین میزان آلودگی مربوط به CMV (٤٦٪)، سپس ZYMV (۳۵٪)، WMV (۲٦٪) و CGMMV (۸٪) در نمونه‌های جمع‌آوری شده بودند. در کل، ٦۳٪، ۵۰٪، ٤۰٪، ۹۰٪، ۷۵٪ و ۸۰٪ به ترتیب از میزبان‌های لوبیا سبز، خیار، بادمجان، کدو، گوجه‌فرنگی و هندوانه حداقل به یک ویروس آلوده بودند. CMV، ZYMV و WMV در اکثر مناطق نمونه‌برداری ردیابی شدند، در حالی‌که CGMMV محدود به شمال‌غرب کشور بود. آلودگی‌های مخلوط دوتایی در ۳۰ نمونه و سه‌تایی در ۸ نمونه از نمونه‌های مورد بررسی ردیابی شدند. آنالیز برش آنزیمی *Msp*I روی ۱۳ قطعه تکثیر شده در PCR با آغازگرهای اختصاصی ژن پروتئین پوششی CMV، حاکی از به دست آمدن پروفایل اختصاصی توصیف شده برای زیرگروه یک این ویروس (قطعات حدود ۵۳۲ و ۳۳۵ جفت باز) برای اغلب جدایه‌ها بود. بر اساس منابع موجود، این اولین گزارش از ردیابی ZYMV و WMV از هندوانه، گوجه‌فرنگی، بادمجان و لوبیاسبز از غرب و شمال‌غرب و اولین گزارش ZYMV از لوبیا سبز از ایران می‌باشد.

کلیدواژه: *آلودگی مخلوط، ویروس‌های صیفی، آرتی-پی‌سی*

---

* بخشی از پایان‌نامه کارشناسی ارشد نگارنده اول، ارایه شده به دانشگاه کردستان

** مسئول مکاتبات، پست الکترونیکی: m.hajizadeh@uok.ac.ir

۱- دانشجوی سابق کارشناسی ارشد و استادیار بیماری شناسی گیاهی، دانشکده کشاورزی، دانشگاه کردستان

۲- استادیار بیماری شناسی گیاهی، دانشکده کشاورزی، دانشگاه زنجان

Detection and identification of four vegetable fruit viruses in west and northwest of Iran

K. Mohammadi[1], M. Hajizadeh[1*], and D. Koolivand[2]

(Received: 18.2.2016; Accepted: 12.6.2016)

Abstract

Field surveys were conducted to detect the incidence of viruses in major vegetable fruit growing areas in west and northwest of Iran. A total of 100 leaf samples from various vegetable fruit crops were analyzed for *Cucumber mosaic virus* (CMV), *Cucumber green mottle mosaic virus* (CGMMV), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV) by RT-PCR using specific primers for each virus. Among the collected samples, the highest incidence was for CMV (46%), followed by ZYMV (35%), WMV (26%) and CGMMV (8%). Overall, 63.3%, 50%, 40%, 90%, 75% and 80% of green bean, cucumber, eggplant, squash, tomato and watermelon samples, respectively, were positive for at least one virus. CMV, ZYMV and WMV were identified in most regions, whereas CGMMV was found only in East Azerbaijan province. Mixed infections of two and three viruses in various combinations were detected in 30 and 8 samples, respectively. Restriction analysis with *Msp*I (*Hpa*II) on 13 PCR products of CMV coat protein revealed a previously described CMV subgroup I (S-I) specific profile (537 and 335 bp fragments) for the isolates. To the best of our knowledge, this is the first report of WMV and ZYMV from watermelon, tomato, eggplant and green bean in the West and Northwest regions of Iran and first report of ZYMV infecting green bean (*Phaseolus vulgaris* L.) in Iran.

Keywords: Vegetable viruses, mixed infection, RT-PCR

* Corresponding Author: m.hajizadeh@uok.ac.ir
1. MSc. Student and Assis. Prof. of Plant Pathology, College of Agriculture, University of Kurdistan, Sanandaj, Iran.
2. Assis. Prof. of Plant Pathology, College of Agriculture, University of Zanjan, Zanjan, Iran.

Introduction

Vegetable fruit crops including green bean (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.), eggplant (*Solanum melongena* L), squash (*Cucurbita pepo* L.), tomato (*Lycopersicum esculentum* L.) and watermelon (*Citrullus lanatus* L.), are the most popular crops worldwide. According to FAOSTAT (2012), Iran is the 2nd, 3rd, 3rd, 5th and 6th largest producer of cucumber, watermelon, eggplant, squash and tomato, respectively, in the world. In the west and northwest of Iran, the climate is favorable for the growth of popular vegetable fruit. However, these crops are prone to attack by viruses, especially aphid-borne viruses resulting in heavy economic losses (Yoon *et al.* 2008; Ullman *et al.* 1991). Infections of *Cucumber mosaic virus* (CMV), *Cucumber green mottle mosaic virus* (CGMMV), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV) in these crops have been frequently reported from many places of the world (Yuki *et al.* 2000; Abou-Jawdah *et al.* 2000; Ayo-John *et al.* 2014). There have been a number of reports on the diagnosis of major viruses of vegetable fruit crops in Iran using biological, serological and molecular methods (Weidmann & Mostafawy 1972; Ghorbani 1988; Sokhandan-Bashir *et al.* 2006; Bananej & Vahdat 2008; Moradi & Jafarpour 2010; Nematollahi *et al.* 2012). Despite the vast vegetation of vegetable fruit crops in the west and northwest of Iran, little information on virus infections in this area is available (Bananej & Vahdat 2008).

The objective of this survey was to determine single or mixed infections of CMV, CGMMV, WMV and ZYMV in vegetable fruit crops. CMV, the type member of the genus *Cucumovirus* (the family *Bromoviridae*) has a wide host range and has been reported from 1325 plant species in 100 families from mono and dicotyledonous plants (Garcia-Arenal & Palukaitis 2008). CGMMV belongs to the genus *Tobamovirus* in the family *Virgaviridae* (Adams *et al.* 2009), is limited to cucurbitaceous plants and is transmitted mechanically, seed, pollen and transplants (Liu *et al.* 2013; Varveri *et al.* 2002). ZYMV and WMV belong to genus *Potyvirus* (the family *Potyviridae*) (Desbiez & Lecoq 1997; Purcifull *et al.* 1984). These viruses are efficiently transmitted by aphids such as *Myzus persicae* in a stylet-borne, non-persistent manner (Lisa & Lecoq 1984). Diseases caused by these viruses are often the most destructive and difficult to control in vegetable fruit crops. Detection of viruses in these crops is an important step for virus disease management.

Materials and Methods

Sample collection

During the summer of 2013 and 2014, one-hundred leaf samples from vegetable fruit crops including cucumber, eggplant, green bean, squash, tomato and watermelon exhibiting viral-like symptoms were collected from several locations of northwest (East and west Azerbaijan provinces) and West (Kurdistan and Kermanshah provinces) of Iran.

RNA extraction and RT-PCR

Total RNA was extracted from each leaf sample as described by Rowhani *et al.* (1993) with minor modifications. Two ml extraction buffer (91 mM K2HPO4, 30 mM KH2PO4, 292 mM sucrose, 0.22 mM bovine serum albumin, fraction V, 0.8 mM polyvinyl pyrrolidone 25, 30 mM ascorbic acid, pH 7.6) was added to homogenize 200 mg of leaf tissue. The homogenized sample was transferred to a 1.5 ml eppendorf tube and centrifuged at 1000 g for 5 minutes. The aqueous phase was transferred to a fresh tube and centrifuged at 16,800 g for 20 minutes. The resultant pellet was suspended in 200 µl Tris buffer (10 mM EDTA, 50 mM Tris, pH 8, 0.1% 2-mercapto-ethanol) and after adding 25 µl of 10% SDS, it was incubated at 60°C for 20 minutes. Then, 80 µl of 5 M potassium acetate was added to the mixture and kept on ice for 30 minutes. After centrifugation at 16,800 g for 15 minutes, the supernatant was collected and transferred to a fresh tube, followed by adding 0.1 volume of 3 M sodium acetate and an equal volume of cold isopropanol. This was stored at -20°C for 1 hr. The tubes were again centrifuged at 16,800 g for 20 minutes and the resulting pellet was washed with 70% ethanol. The pellet was dried and suspended in 40 µl RNase-free sterile distilled water.

Reverse transcription (RT) reactions were performed in 10µl final volume containing 1µl (0.2 µg/ml) random hexamer primer, 4µl aliquot of

extracted total RNA and 5µl cDNA synthesis

Table 1. PCR primers used to detect Cucumber green mottle virus (CGMMV), Cucumber mosaic virus (CMV), Watermelon mosaic virus (WMV) and Zucchini yellow mosaic virus (ZYMV) in vegetable fruit hosts.

Virus	Primer	Sequence (5´- 3´)	Amplicon length (bp)	AT*	Amplified position	References
CMV	CMV(CPf)	GCTTCTCCGCGAG	867	52	CP/1148-2015	Rizos *et al.*, 1992
	CMV (CPr)	GCCGTAAGCTGGATGGAC				
CGMMV	CGMMV (CPf)	GAAGAGTCCAGTTCTGTTTC	523	50	CP/5735-6258	Yoon *et al.*, 2008
	CGMMV (CPr)	ACCCTCGAAAACTAAGCTTTC				
WMV	WMV (CPf)	CCGCAAAACTCACAAACTGG	657	52	CP/9024-9681	Shimomoto and Takeuchi., 2006
	WMV (CPr)	CCTGCTGTTAATTCCCGCG				
ZYMV	ZYMV (f)	TCTCATCAGCAATTCGCCTC	432	50	CP/8830-9262	Shimomoto and Takeuchi., 2006
	ZYMV (r)	AGAAACATTGCTAAGGGCTG				

* Annealing Temperature (AT)

solution from the kit (HyperScript™ Reverse Transcriptase, GeneAll, Seoul, Korea). The mixture was incubated at 55ºC for 60 min in a BioRad thermocycler (BioRad, USA).

PCR reactions were done using PCR master mix (Amplicon, GeneAll, Seoul, Korea) (Tris-HCl pH 8.5, $(NH4)_2SO_4$, 3 mM $MgCl_2$, 0.2% Tween 20, 0.4 mM dNTPs, 0.2 Units/µl Ampliqon *Taq* DNA polymerase), in the above thermocycler. The PCR reaction contained 1X Kapa buffer A (+Mg), 0.4 µM forward and reverse primer (Table 1), 0.2 µM dNTPs, 1X cresol loading dye (20% w/v sucrose, 1 mM cresol red), 4.75 µl RNase-free sterile distilled water and 1µl cDNA in a 12.5 µl reaction.

The cycling conditions of the PCR were 94°C for 2 min for the initial denaturation, followed by 35 cycles of 94°C for 30 s, annealing temperature (as shown in Table 1) for 30 s, 72°C for 1 min for the extension and a final elongation step for 7 min at 72°C. PCR products were electrophoresed on a 1.2% (w/v) agarose gel (containing 0.5 µg/µl EtBr) in 1X TAE buffer. Electrophoresis was carried out at 80 V for approximately 1 h. Fragments were visualized with a UV transilluminator and photographed in a gel documentation (UVP, USA) apparatus.

Restriction analysis

To determinate the CMV subgroup lineage by molecular purification using a restriction enzyme and PCR-RFLP (Rizos *et al.* 1992), the CMV CP cDNAs from 13 isolates B11, C35G, Z2, Z3, B10, E2, C10, C8, C41, H7, 23M, B21, ZMK4 were digested by the 4-bp-cutter enzyme *Msp*1 (CinnaClon, Iran). The digestions were done according to the manufacturer's instruction. Approximately 100 ng of each CMV CP PCR product was digested in 1X restriction buffer and 5 unit of the enzyme. The reactions were incubated at 37 °C for 2h, followed by electrophoresis in 1.5% agarose gel as described previously.

Cloning, sequencing and sequence analysis

To verify the PCR results, amplified fragments were ligated into the pTG19 vector (CinnaClon, Iran) by T_4 DNA ligase (CinnaClon, Iran) according to the manufacturer's instruction with the minor modification using 25 ng of the vector and about 10 ng of the PCR product. The ligation mixture was incubated at 16 ºC for 1h and then at 22 ºC overnight as instructed by the manufacturer. The recombinant vector was introduced into competent *Escherichia coli* strain DH5α cells and transformed cells were selected on LB plate containing ampicillin (100 µg/ml), IPTG (50 µl of 0.1 M) and X-Gal (10 µl of 50 mg/ml) as described elsewhere (Sambrook & Russell, 2001). Three white colonies for each virus growing on the LB selection plate were grown in 5 ml LB medium, and kept overnight at 37 ºC before subjecting to plasmid purification by the alkaline lysis method (Ish-Horowicz & Burke 1981) or using Exprep plasmid SV (GeneAll, Seoul, Korea). Purified plasmids were digested with *Bam*HI restriction enzyme, followed by electrophoresis on

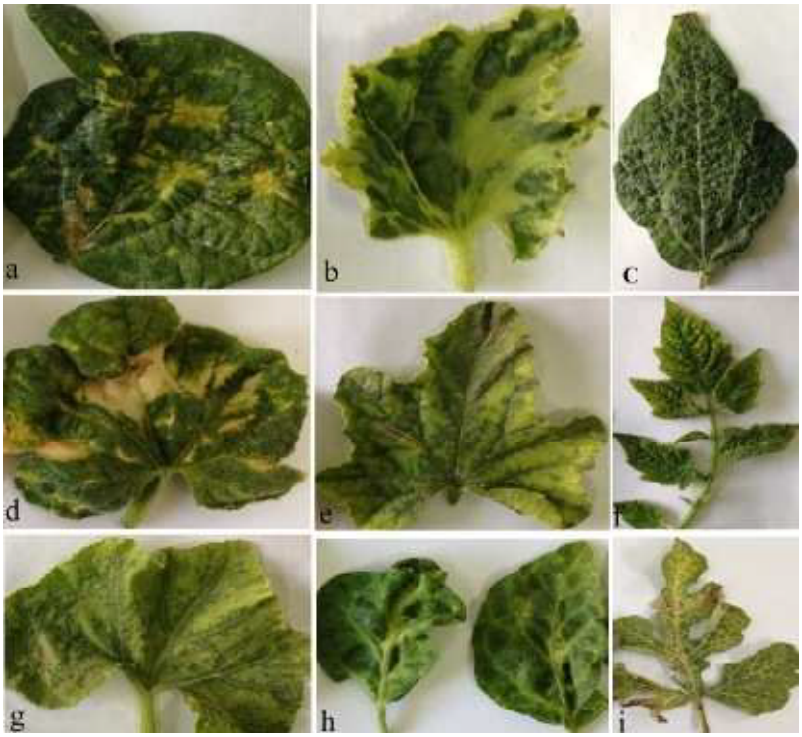

Figure 1. Fruit vegetables infected with different viruses: (A) Green bean(B_{21}) triple infected with *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV); (B) Cucumber(C_{47}) double infected with CMV and ZYMV; (C) Green bean (B22) double infected with CMV and ZYMV; (D) Squash (Z_1) double infected with CMV and ZYMV;(E) Watermelon (W_5) double infected with WMV and ZYMV (F) Tomato (T_{10}) double infected with WMV and ZYMV; (G) Squash (Z_{15}) infected with ZYMV; (H) Eggplant (E_2) infected with CMV; and (I) Watermelon (W_{23}) double infected with CMV and WMV.

1.5% agarose. Each colony carrying the cloned CP cDNA was isolated, purified and sequenced with M13 universal primers. Recombinant plasmids were sequenced by Macrogen Inc. (Seoul, South Korea) and compared to in GenBank isolates using the BLASTn program (Altschul *et al.* 1990).

Results and Discussion

Symptoms of collected samples

Generally, typical virus symptoms were observed in the fields including mild to severe mosaic, leaf wrinkling, vein banding, yellowing and leaf deformation as previously described for CMV (Kaper & Waterworth 1981), CGMMV (Francki 1996), WMV (Purcifull *et al.* 1984) and ZYMV (Desbiez & Lecoq 1997) (Fig. 1). Due to mixed infections, it was difficult to identify the symptoms of a particular virus species in the field and hence we initiated a diagnosis based on the molecular methods.

Detection and distribution of viruses in different vegetable fruits

In RT-PCR, fragments of the expected size of 523, 867, 657 and 432 bp from the coat protein genes of CGMMV, CMV, WMV and ZYMV, respectively were amplified (Table 1; Fig. 2). CMV, WMV and ZYMV were detected in symptomatic samples collected from both regions (Northwest and West) in all hosts, while CGMMV was only detected in 8% of squash samples collected in the Northwest during two years study (Table 2), indicating a low incidence of CGMMV in vegetable fruit.

CMV was the most prevalent virus, being detected in 46 samples collected from different hosts in both regions. The incidence of CMV in the Northwest and West provinces was 82.5% and 26.7% of the collected samples, respectively. CMV was detected in 100% of cucumber and tomato, 73.7% of squash and 50% of watermelon samples in northwest region while, the infection rate within collected samples in west region was 50%, 40%,

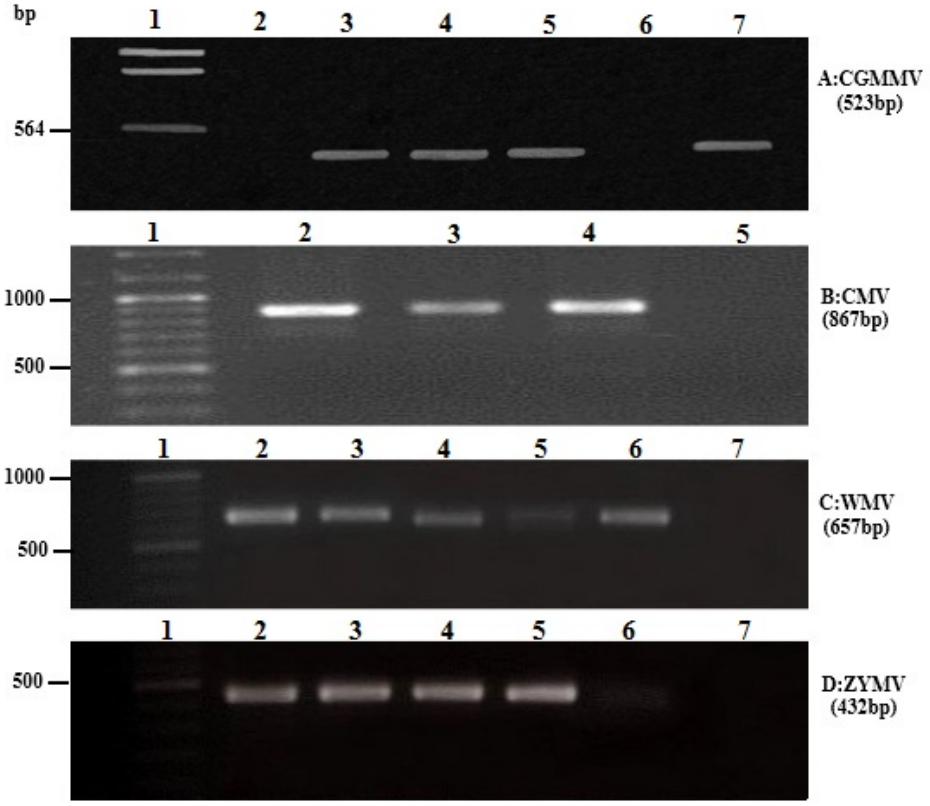

Figure 2. Electrophoretic patterns of PCR products on 1.2 % agarose gel showing infection of samples to A) *Cucumber mosaic virus*, B) *Watermelon mosaic virus*, C) *Zucchini yellow mosaic virus* and D) *Cucumber green mottle mosaic virus*. Lane 1) 1kb DNA ladder.

Table 2- Incidence of viruses in individual vegetable fruit crops from symptomatic samples collected in west and northwest of Iran during 2013 to 2014.

Region, host species (scientific name)	Common name	Samples collected	Number of samples positive by RT-PCR*			
			CGMMV	CMV	WMV	ZYMV
Northwest of Iran**						
Citrullus lanatus L.	Watermelon	4	0	2	0	3
Cucumis sativus L.	Cucumber	3	0	3	0	0
Cucurbita pepo L.	Squash	19	8	14	2	12
Lycopersicum esculentum L.	Tomato	14	0	14	0	0
Total		40	8(20%)	33(82.5%)	2(5%)	15(37.5%)
West of Iran***						
C. lanatus L.	Watermelon	6	0	3	4	2
C. sativus L.	Cucumber	21	0	4	7	5
C. pepo L.	Squash	11	0	4	6	7
L. esculentum L.	Tomato	6	0	0	1	1
Phaseolus Vulgaris L.	Green bean	11	0	3	6	5
S. melongena L	Eggplant	5	0	2	0	0
Total		60	0	16(26.6%)	24(40%)	20(33.3%)
Total		100	8(8%)	49(49%)	26(26%)	35(35%)

*RT-PCR= reverse transcriptase-polymerase chain reaction, ** west and east- Azerbaijan provinces, ***Kurdistan and Kermanshah provinces: CGMMV= *Cucumber green mottle mosaic virus*, CMV= *Cucumber mosaic virus*, WMV= *Watermelon mosaic virus*, ZYMV= *Zucchini yellow mosaic virus*.

36%, 27% and 19% in watermelon, eggplant, squash, green bean and cucumber, respectively. CMV was not found in tomato in the West region (Table 2).

WMV was detected in 26 samples (2 samples in the Northwest and 24 samples in the West) collected from different hosts in both regions. The infection rate of WMV was only 10.5% in squash in the Northwest, while it was not detected in other vegetable fruit crops. Among the collected samples, in the West region, WMV was detected in all vegetable fruit except eggplant with infection rate of 66.7%, 54.5%, 54.5% 33.3% and 16.7% in watermelon, green bean, squash, cucumber and tomato, respectively (Table 2).

ZYMV was detected in 35 samples (15 samples from the Northwest and 20 samples from the West regions) from different hosts. This virus had the second highest incidence in our survey. The infection rate of ZYMV within collected samples was 75% and 63% in watermelon and squash, respectively, in the Northwest region while this virus was not detected in cucumber and tomato. In the West region, the infection rate of ZYMV within collected samples was 63.6 in squash, 45.5% in green bean, 33.3 in watermelon, 23.8 in cucumber and 16.7% in tomato. In contrast, none of the eggplant samples were positive for ZYMV (Table 2).

We found that CMV, WMV and ZYMV were widespread in both regions and, therefore, they may incur the highest economic impact on vegetable fruit. The occurrence of CMV, WMV and ZYMV on squash and cucumber from northwest and west of Iran was reported before (Bananej & Vahdat 2008), but this study extends our knowledge on the natural infection of CMV, WMV and ZYMV using additional samples and different hosts for these viruses in these regions. These hosts include watermelon, tomato, eggplant and green bean, the latter being a new host for ZYMV in Iran. There was a high incidence of CMV, WMV and ZYMV, but CGMMV was detected only in squash in the Northwest region as previously reported (Bananej & Vahdat 2008). None-persistent mode of transmission of CMV, WMW and ZYMV by many aphids facilitates rapid spread of the virus (Garzo *et al.* 2004). We failed to detect CMV, CGMMV, WMV and ZYMV in a number of symptomatic samples and we speculate the infection by other viruses or viroids.

Mixed infection of viruses

Mixed infections were recorded in 38 samples (38%). About 30 and 8 samples were simultaneously infected with two and three viruses, respectively and no sample was infected with four viruses. In details CGMMV, CMV, WMV and ZYMV occurred in various combinations in 8%, 56.5%, 100% and 77.1% of the samples, respectively. Furthermore, 30% of samples showed double infections by WMV and ZYMV (12%), CMV and WMV (7%), CMV and ZYMV (6%) and CGMMV and CMV (5%). No mixed infection was ever found in eggplant (Table 3).

Most triple infections were detected in green bean and squash samples. In details, CGMMV, CMV and ZYMV were detected in 3% and CMV, WMV and ZYMV were found in 5% of samples (Table 3). It is noteworthy that the triple infection of CMV, WMV and ZYMV showed severe symptoms compared to other samples. The result showed that three major viruses are distributed in most regions in the West of Iran. Altogether, it was not possible to associate any of the virus combinations with a specific symptom. In co-infections involving CMV and WMV/or ZYMV enhanced symptom expression was observed (data not shown).

Mixed infections of heterologous viruses such as *Potyviridae* family together with viruses from other families such as CMV appear to cause severe synergistic effects on virulence (Moradi & Jafarpor 2010; Wang *et al.* 2002). However, it was not possible to associate any virus combination with a specific type of symptom. The high incidence of CMV, WMV and ZYMV and co-infections especially of CMV with WMV and/or ZYMV in all samples of vegetable fruit in the West and Northwest of Iran, suggest that these viruses represent a potential threat to these crops in Iran.

CMV subgroups

*Msp*I restriction on CMV PCR products amplified from the isolates B11, C35G, Z2, Z3, and B10 resulted two DNA fragments with sizes of approximately 532 and 335 (Fig. 3) was consistent with the *Msp*I restriction map of CMV subgroup-I (S-I) strains (Rizos *et al.*, 1992). However, Additional fragment(s) were produced on isolates E2, H7, 23M, C8, C41 and C40 (for example; Fig.

Table 3- Number of mixed infection samples in vegetable fruit crops during 2013 to 2014 as determined by RT-PCR*.

Region, crops	Mixed/total	Double infections				Triple infection	
		CGMMV+CMV	CMV+WMV	CMV+ZYMV	WMV+ZYMV	CGMMV+CMV+ZYMV	CMV+WMV+ZYMV
Northwest of Iran**							
Cucumber	0/3	0	0	0	0	0	0
Squash	14/19	5	1	4	1	3	0
Tomato	0/14	0	0	0	0	0	0
Watermelon	1/4	0	0	1	0	0	0
Total	15/40	5(12.5%)	1(2.5%)	5(12.5%)	1(2.5%)	3(7.5%)	0
West of Iran***							
Cucumber	7/21	0	2	0	5	0	0
Eggplant	0/5	0	0	0	0	0	0
Green bean	5/11	0	1	O	2	0	2
Squash	7/11	0	1	1	3	0	2
Tomato	1/6	0	0	0	1	0	0
Watermelon	3/6	0	2	0	0	0	1
Total	23/60	0	6(10%)	1(1.7%)	11(18.3%)	0	5(8.3%)
Total	38/100	5(5%)	7(7%)	6(6%)	12(12%)	3(3%)	5(5%)

*RT-PCR= reverse transcriptase-polymerase chain reaction, ** West and East- Azerbaijan provinces, ***Kurdistan and Kermanshah provinces.

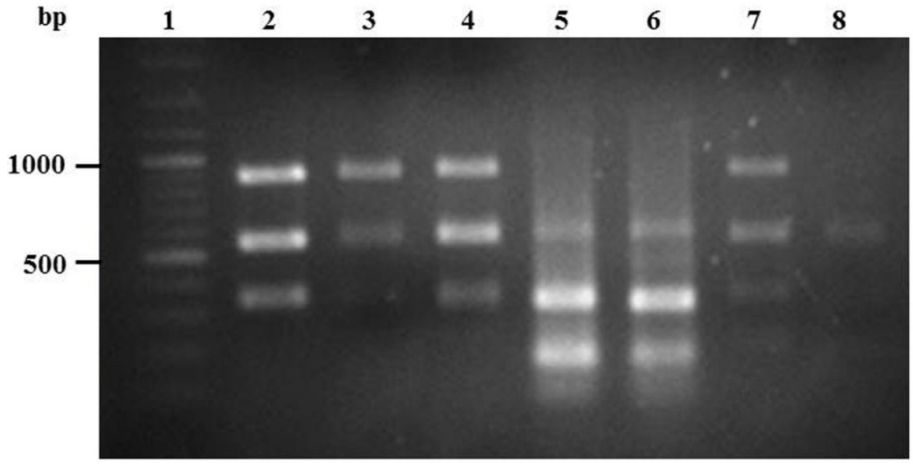

Figure 3. *Msp*I digestion profile on CMV cDNA from isolates; lane1) 1kb DNA ladder, lanes 2-8) Z3, B11,C35G, E2, E7, B10 and Z2, respectively.

3, lanes 5 and 6). These results suggested a possible combined *Msp*I profile of two variants, one with an S-I-specific profile (only one *Msp*I site), and the other with additional *Msp*I sites. The combined *Msp*I profiles were resulted of mixed CMV variants. Such mixed replicating virus populations occur because of high mutation rates in the genomes, which account for virus evolution. Viruses with RNA genomes, such as CMV, evolve rapidly because of replication by error-prone RNA polymerases and short generation period (Sokhandan-Bashir *et al.* 2006). *Msp*I digestion on isolates C10 and ZMK4 were produced fragments which not consistent with *Msp*I profiles map of known CMV subgroups I and II (Rizos *et al.*, 1992) and need to further investigation (data not shown). In some isolates, an intact 867 bp fragment was also obtained due to incomplete digestion by *Msp*I restriction enzyme (Fig. 3, lanes 2, 3, 4 and 7).

Cloning and sequencing of the PCR products

The amplified fragment from CMV cDNA (Z3 isolate) of squash sample showed a size of 867 nt, which was deposited in GenBank and assigned the

accession No. KP751210. It was 92.5% identical to the CMV reference sequence (NC_001440) from the USA (Owen *et al.*, 1990). BLASTn analyses showed that it had a sequence identity of 92.7-94.7% with CMV variants previously reported from Iran (Sokhandan-Bashir *et al.* 2006; 2008), 92.5% to strain fny (U20668), 90.6% to strain SD (AB008777) and 75.5% to strain Q (M21464). Phylogenetic analysis based on CMV CP sequences including previously reported GenBank accessions showed that isolate Z3 belonged to CMV S-IA.

A sequenced clone from WMV in squash from Iran (Z2) in this study (accession No. KP751208) had a size of 657 nt, with 98% nucleotide homology with the reference sequence (GenBank accession No. NC_006262) from the USA (Desbiez & Lecoq, 2004). BLASTn analysis showed that KP751208 had 93-99% identity with a WMV variant previously reported from Iran (EU660579, EU660583, EU667643, JN831650) and 93-98% identical to other corresponding sequences already available in the GenBank.

The ZYMV isolate from green bean had a size of 432 nt (accession No. KP751207) and was 94-99% identical to other isolates previously reported from Iran (FJ705271, FJ705267, FJ705272, JN183062). BLASTn analysis showed that our isolate was 91% identical to the ZYMV reference strain (NC_003224) from Taiwan (Lin *et al.* 2001) and 91-99% identical to sequences already deposited in the GenBank.

To the best of our knowledge, this is first report of WMV and ZYMV from watermelon, tomato, eggplant and green bean in the West and Northwest regions of Iran and first report of ZYMV infecting green bean (*Phaseolus vulgaris* L.) in Iran.

Acknowledgment

We would like to thank Dr. Nemat Sokhandan-Bashir (University of Tabriz) and Dr. Nuredin Habili (University of Adelaide) for careful review of this paper.

References

Abou-Jawdah Y., Sobh H., Fayad A., Lecoq H., Delecolle B. and Trad-Ferre J. 2000. Cucurbit yellow stunting disorder virus: A new threat to cucurbits in Lebanon. Journal of Plant Pathology 82: 55-60.

Adams M. J., Antoniw J. F. and Kreuze J. 2009. Virgaviridae: a new family of rod-shaped plant viruses. Archives of Virology 154: 1967-1972.

Altschul S. F., Gish W., Miller W., Myers E.W. and Lipman D. J. 1990. Basic local alignment search tool. Journal of Molecular Biology 215: 403-10.

Ayo-John E. I., Olorunmaiye P. M., Odedara O. O., Dada O. B., Abiola K. O. and Oladokuni, J. O. 2014. Assessment of field-grown cucurbit crops and weeds within farms in south-west Nigeria for viral diseases. Notulae Scientia Biologicae 6:321-325.

Bananej K. and Vahdat A. 2008. Identification, distribution and incidence of viruses in field-grown cucurbit crops of Iran. Phytopathologia Mediterranea 47: 247–257.

Desbiez C. and Lecoq H. 1997. *Zucchini yellow mosaic virus*. Journal of Plant Pathology 46: 809–829.

Desbiez C. and Lecoq H. 2004. The nucleotide sequence of *Watermelon mosaic virus* (WMV, *Potyvirus*) reveals interspecific recombination between two related potyviruses in the 5' part of the genome. Archives of Virology 149: 1619-1632.

F.A.O. 2012. F.A.O. Statistical Databases. Food and Agriculture Organization of the United Nations.

Francki R. I. B. 1996. *Cucumber green mottle mosaic tobamovirus*. In: A A. Brunt, K. Crabtree, M.J. Dallwitz, A.J. Gibbs, L. Watson and E.J. Zurcher (Eds). Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: August 20. [http://biology.anu.edu.au/Groups/MES/vide/].

Garcia-Arenal F. and Palukaitis P. 2008. *Cucumber mosaic virus*, pp. 614-619. In: B. W. J. Mahy and M. H. V. Van Regenmortel (Eds). Encyclopedia of Virology. 3rd ed., Oxford: Academic Press.

Garzo E. I., Duque M. and Fereres A. 2004. Transmission efficiency of different non-persistent viruses infecting melon by four aphid species. Spanish Journal of Agriculture Research 2: 369-376.

Ghorbani S. 1988. Isolation of Zucchini yellow mosaic virus in the Tehran province. Iranian Journal of Plant Pathology 24: 13-15.

Ish-Horowicz D. and Burke J. F. 1981. Rapid and efficient cosmid cloning. Nucleic Acids Research 9: 2989.

Kaper J. M. and Waterworth H. C. 1981. Cucumoviruses, pp.257-332. In: E. Kurstak (Ed). Handbook of Plant Virus Infections: Comparative Diagnosis. Elsevier/North-Holland Biomedical Press, Amsterdam, The Netherlands.

Lin S. S., Hou R. F. and Yeh S. D. 2001. Complete genome sequence and genetic organization of a Taiwan isolate of *Zucchini yellow mosaic virus*. Botanical Bulletin of Academia Sinica 42: 243-250.

Lisa V. and Lecoq H. 1984. *Zucchini yellow mosaic virus*. Descriptions of Plant Viruses, no. 282. Commonwealth Mycological Institute and Association of Applied Biologists, Kew, England.

Liu H. W., Luo L. X., Li J. Q., Liu P. F., Chen X. Y. and Hao J. J. 2013. Pollen and seed transmission of *Cucumber green mottle mosaic virus* in cucumber. Journal of Plant Pathology 63: 73-77.

Moradi Z. and Jafarpour B. 2010. Mixed infections of *Watermelon mosaic potyvirus* and *Cucumber green mottle mosaic tobamovirus* in Cucurbit hosts. Journal of Crop Protection 2: 353-365.

Nematollahi S., Sokhandan-Bashir N., Rakhshandehroo F. and Hamid Zamanizadeh R. 2012. Phylogenetic analysis of new isolates of *cucumber mosaic virus* from Iran on the basis of different genomic regions. The Plant Pathology Journal 28: 381-389.

Owen J., Shintaku M., Aeschleman P., Ben Tahar S. and Palukaitis, P. 1990. Nucleotide sequence and evolutionary relationships of cucumber mosaic virus (CMV) strains: CMV RNA 3. Journal of General Virology 71: 2243-2249.

Purcifull D., Hiebert E. and Edwardson J. 1984. Watermelon mosaic virus 2. CMI/AAB Descriptions of Plant Viruses. No 293 Preveza, Greece, 115. Commonwealth Mycological Institute and Association of Applied Biologists, Kew, England, UK.

Rizos H., Gunn L. V., Pares R. D. and Gillings R. M. 1992. Differentiation of *Cucumber mosaic virus* isolates using the polymerase chain reaction. Journal of General Virology 73: 2099−2103.

Rowhani A., Chay C., Golino D. A. and Falk W. 1993. Development of a polymerase chain reaction technique for the detection of *Grapevine fanleaf virus* in grapevine tissue. Journal of Phytopathology 83: 749-753.

Sambrook J, Russell D. W. 2001. Molecular cloning: a laboratory manual. 3rd ed., Cold Spring Harbor Laboratory Press, New York.

Shimomoto Y. and Takeuchi S. 2006. Simultaneous detection of three potyvirus species from Cucurbitaceae crops by multiplex RT-PCR. Japanese Journal of Phytopathology 72: 146-149.

Sokhandan-Bashir N., Kolhar M. R. and Zarghani S. N. 2006. Detection, differentiation and phylogenetic analysis of *Cucumber mosaic virus* isolates from cucurbits in the northwest region of Iran. Virus Genes 32: 277−288.

Sokhandan-Bashir N., Nematollahi S. and Torabi E. 2008. *Cucumber mosaic virus* subgroup IA frequently occurs in the northwest Iran. Acta Virologica 52: 237−242.

Ullman D. E., Cho J. J. and German T. L. 1991. Occurrence and distribution of cucurbit viruses in the Hawaiian Islands. Plant Disease 75: 367-370.

Varveri C., Vassilakos N. and Bem F. 2002. Characterization and detection of *Cucumber green mottle mosaic virus* in Greece. Phytoparasitica 30: 493-501.

Wang Y. Z., Gaba, V., Yang, J., Palukaitis, P. and Gal-On, A. 2002. Characterization of synergy between *Cucumber mosaic virus* and potyviruses in cucurbit hosts. Journal of Phytopathology 92: 51–58.

Weidmann H. L. and Mostafawy M. 1972. *Watermelon mosaic virus* type 2 in Iran and its transmission by different aphid species. Iranian Journal of Plant Pathology 8: 20.

Yoon J.Y., Choi G. S., Choi S. K., Hong J. S., Choi J. K., Kim W., Lee G. P. and Ryu K. H. 2008. Molecular and biological diversities of *Cucumber green mottle mosaic virus* from Cucurbitaceous crops in Korea. Journal of Phytopathology 156: 408-412.

Yuki V. A., Rezende J. A. M., Kitajima E. W., Barroso P. A. V., Kuniyuki H. and Groppo G. A. 2000. Occurrence, Distribution, and Relative Incidence of Five Viruses Infecting Cucurbits in the State of Sao Paulo, Brazil. Plant Disease 84: 516-520.