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Identification, distribution and incidence of viruses in field-grown cucurbit crops of Iran

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Summary. A survey of viruses in the major cucurbit-growing areas of 17 provinces in Iran was conducted in 2005 and 2006. A total of 1699 leaf samples were collected from melon, squash, cucumber and watermelon plants showing various virus-like symptoms. Screening for 11 cucurbit viruses by double-antibody sandwich ELISA (DAS-ELISA) or RT-PCR, found that 71% of the samples were infected by at least one virus, of which *Cucurbit aphid-borne yellows virus* (CABYV) was the most common overall, occurring in 49, 47, 40, and 33% of cucumber, squash, melon, and watermelon samples respectively. The second most common virus on melon and watermelon was *Watermelon mosaic virus* (WMV) (incidence 30–33%); on cucumber, *Cucumber mosaic virus* (CMV)(33%); and on squash, *Zucchini yellow mosaic virus* (ZYMV)(38%). To our knowledge, this is the first report of *Melon necrotic spot virus* (MNSV) and *Zucchini yellow fleck virus* (ZYFV) in Iran. Mixed infections occurred in 49% of symptomatic samples. Mixed infections were relatively frequent in squash (58%) and melon (55%). The most frequent double infections were WMV+CABYV and ZYMV+CABYV in melon, squash and cucumber, followed by WMV+ZYMV. In watermelon, the most frequent double infection was WMV+ZYMV, followed by WMV+CABYV. The high frequency of CABYV, WMV and ZYMV in the samples assayed on all four cucurbit crops and in all areas surveyed, as well as the detection of *Watermelon chlorotic stunt virus* (WmCSV) and *Cucumber vein yellowing virus* (CVYV) in northern and southern Iran, suggest that these viruses represent a potential threat to cucurbit crops in Iran.

Key words: cucurbit viruses, DAS-ELISA, RT-PCR, mixed infections.

Introduction

The main cultivated cucurbit species: melon (*Cucumis melo* L.), cucumber (*C. sativus* L.), squash (*Cucurbita* sp.), and watermelon (*Citrullus lanatus* L.) are important vegetable crops worldwide, especially in developing countries. Viral diseases of cucurbit crops cause important economic losses throughout the world. More than 35 viruses have been isolated from cucurbits (Provvidenti, 1996). These viruses cause complex and dynamically changing problems, as was described by Nameth *et al*. (1986). *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV), and *Papaya ringspot virus type W* (PRSV-W) are the three most frequent and economically important cucurbit viruses worldwide (Lecoq *et al.,* 2001). ZYMV is a particularly notorious virus (Lecoq *et al.,* 1983) and, since its first appearance in Italy and France in 1973 and 1979 respectively, it has been reported in 50 countries both in traditional and in intensive growing conditions (Desbiez and Lecoq, 1997).

Cucurbit aphid-borne yellows virus (CABYV) is one of several viruses causing yellowing symptoms in cucurbit crops. CABYV was first described in 1992 in France and early infections with CABYV may lead to a ca. 50% yield loss in cucumber, and 40% in melon (Lecoq 1992). CABYV is a member of the genus *Polerovirus* in the family *Luteoviridae* (Mayo and D'Arcy, 1999), and causes severe yellowing in cucurbit crops in France (Lecoq *et al*., 1992) and in

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the United States (Lemaire *et al.*, 1993). Typical symptoms in cucumber, melon, squash, and watermelon include yellowing and thickening of the older leaves. CABYV severely reduces yield in melon and cucumber by reducing the number of fruits per plant as a result of a high percentage of flower abortions (40 and 51%, respectively) but it does not alter fruit shape or quality (Dogimont *et al.*, 1996). *Cucumber green mottle mosaic virus* (CGMMV), *Cucumber mosaic virus* (CMV), *Squash mosaic virus* (SqMV), and *Melon necrotic spot virus* (MNSV) have also long been reported to be important viruses infecting cucurbit crops (Lovisolo, 1980).

Cucurbits are major vegetable crops in Iran, ranking first in economic value, second in yield, and third in acreage. With 80,000 hectares under melon cultivation, Iran is the third biggest producer of melon in the world after China and Turkey (Anonymous, 1996). Cucurbit crops occupy 341,058 ha in different growing regions of Iran and are directly seeded and not shipped from one province to another. Almost 75% of cucurbit crop production is from open field-grown crops. Of the approximately 35 viruses reported on the *Cucurbitaceae* worldwide (Provvidenti, 1996; Lovisolo, 1980), ZYMV, WMV, CGMMV, WmCSV, CMV, *Cucumber yellow stunting disorder virus* (CYSDV), PRSV-W, SqMV, *Ourmia melon virus* (OuMV), CABYV, and CVYV have been reported from field-grown cucurbit crops in Iran (Danesh, 1969; Ebrahim-Nesbat, 1972; Ebrahim-Nesbat, 1974; Rahimian and Izadpanah, 1978; Ghorbani, 1986, 1988; Lisa *et al.*, 1988; Parvizy, 1989; Bananej *et al.*, 1998; Keshavarz and Izadpanah, 2004; Bananej *et al.*, 2006a; Bananej *et al.*, 2006b). In these earlier studies only a limited number of samples from a few cucurbit-growing areas were tested and there is only very limited information at the local level on the characterization, distribution and incidence of viruses infecting cucurbits. However, observations indicate that mosaic, yellowing, vein clearing, and leaf and fruit malformation are very frequent in field-grown squash, watermelon, melon and cucumber crops in the country. This report summarizes the findings of a systematic survey carried out during two growing seasons (2005 and 2006), in order to identify and evaluate the distribution and the incidence of viruses infecting four cucurbit crops (melon, squash, cucumber and watermelon) in 17 provinces of Iran. Such information is needed as a first step in developing and implementing effective means of control.

Materials and methods

Surveys and sample collection

Surveys were conducted in open-field melon, squash, cucumber and watermelon crops grown in 17 provinces of Iran in 2005 and 2006 (from June through October in 2005 and 2006, and from November to December in 2006). Samples were obtained from 123 fields randomly selected in each geographical area surveyed (Fig. 1 and Table 1). Selected fields were separated by a minimum of 5 km. Each field was inspected and sampled once, during the mid-developmental stage of the crop. A sample consisted of two to three leaves per plant. Six hundred and forty-six melon, 541 squash, 223 watermelon, and 289 cucumber samples from symptomatic and asymptomatic plants were collected for virus detection (Table 1). From a total of 1699 plants sampled, 1406 had symptoms suggestive of virus infection. As melon and squash are the most cultivated cucurbit crops in Iran and have a high incidence of viral symptoms, most samples were collected from these particular crops. Most of the fields surveyed were planted with local cucurbit cultivars which are directly seeded. The melon cultivars included Gorgab, Mashadi, and Khaghani, the cucumber cultivars Varamin, and Esfahan, and the squash cultivars Varamin, and Mashad. Sugar baby is the most frequent watermelon cultivar cultivated by Iranian farmers.

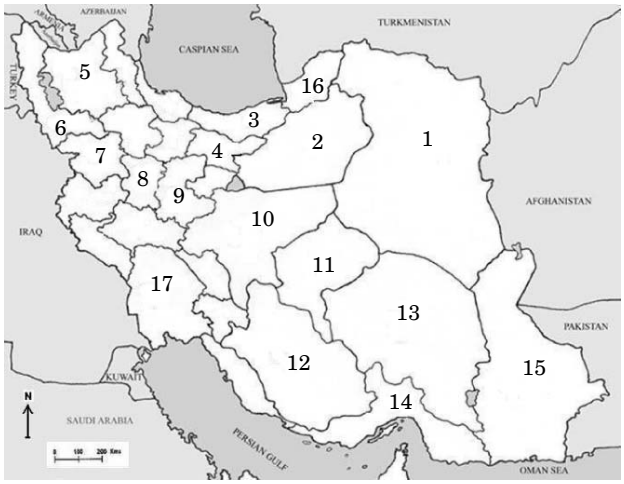

Fig. 1. Map of Iran showing provinces in which surveys were conducted, during the 2005-2006 growing seasons: 1, Khorasan; 2, Semnan, 3, Mazandaran; 4, Tehran; 5, Azarbaijan-E-Sharghi, 6, Azerbaijan-E-Gharbi, 7, Kordestan, 8, Hamedan, 9, Markazi, 10, Esfahan; 11, Yazd; 12, Fars, 13, Kerman; 14, Hormozgan; 15, Sistan-Baluchestan; 16, Golestan and 17, Khozestan.

Table 1. Cucurbit crops surveyed and samples collected by province in Iran.

Year	Province	No. of samples	Cucurbit crop			
			Squash	Melon	Watermelon	Cucumber
2005	Khorasan	125	32	55	16	22
	Semnan	79	15	34	10	20
	Tehran	105	31	43	9	22
	Esfahan	119	39	45	13	22
Subtotal – 2005		428	117	177	48	86
2006	Azarbaijan-E-Sharghi	75	34	29	0	12
	Azarbaijan-E-Gharbi	67	29	24	0	14
	Kordestan	54	19	21	0	14
	Hamedan	91	32	36	13	10
	Markazi	96	30	41	10	15
	Yazd	112	41	35	18	18
	Fars	124	35	50	19	20
	Kerman	110	36	35	24	15
	Hormozgan	79	22	29	16	12
	Sistan-Baluchestan	99	38	30	15	16
	Khozestan	132	38	50	25	19
	Golestan	112	35	44	15	18
	Mazandaran	120	35	45	20	20
Subtotal - 2006		1271	424	469	175	203
Total (2005-2006)		1699	541	646	223	289

Serological tests

The standard DAS-ELISA was performed according to Clark and Adams (1977). IgGs and alkaline phosphatase conjugated IgGs were used for eleven viruses: CABYV, WMV, ZYMV, CMV, SqMV, PRSV-W, WmCSV, CGMMV, CVYV, MNSV, and ZYFV (kindly provided by H. Lecoq; INRA, Avignon, France). Positive controls were provided by H. Lecoq or taken from our collection. Leaf samples were ground in a pre-cooled mortar and pestle with an extraction buffer (PBST: 0.13 M NaCl, 0.003 M KCl, 0.008 M Na_2HPO_4, 0.001 M KH_2PO_4, pH 7.4) containing 0.05% Tween 20 and 0.1% nonfat dry milk and were placed in wells that had been precoated with specific polyclonal antisera diluted in a carbonate buffer (pH 9.6). Plates (Nunc Microwell, Roskilde, Denmark) were incubated at 4°C overnight and washed three times with PBST-Tween 20 buffer. Plates were then coated with alkaline phosphatase conjugated antibody diluted in extraction buffer and incubated for 2 h at 37°C. After washing, *p*-nitrophenyl phosphate in diethanolamine substrate buffer (0.5 mg ml^{-1}, pH 9.8) was added to each well and incubated at room temperature for 30 to 120 min. The reaction was detected colorimetrically at A405 nm using an ELISA reader (MCC-340, Multiscan Labsystem, Finland). Two wells were used per sample. Virus-free cucurbit species grown in insect-proof cages were used as negative controls. Positive and healthy controls were included in all tests. Samples were considered to be positive if the A_{405} nm values were more than three times those of the healthy control.

Reverse transcription-polymerase chain reaction (RT-PCR)

To confirm ZYMV, CVYV, and CABYV identification, total RNA extracts were obtained from leaves of infected plants that were positive in DAS-ELISA,

using TRI-Reagent (Sigma Chemical, St Louis, MO, USA). RNA resuspended in 20 μl DEPC-treated H_2O was heated to 65ºC for 5 min before reverse transcription.

ZYMV and CABYV RT-PCR

The primers used in this study and the expected length of each amplicon are shown in Table 2. Four μl of RNA were submitted to reverse transcription in a final volume of 20 μl, using the ZYMV-CP-3´ (5´-ATGTCGAGTATCACATTTCC-3´, nt position 8756-8775, numbered according to AY188994) (Desbiez *et al.,* 1996), and CABYV-CP (5´-CCYGCAACCGAGGAAGATCC-3´) (Guilley *et al.*, 1994) primers, for 1 h at 42ºC with M-MuLV reverse transcriptase (Fermentas, Vilnius, Lethuania). Two and a half μl of the RT reactions were used for PCR. PCR reactions were carried out in an Eppendorf Mastercycler 5330 with the following conditions: initial denaturation of 94ºC for 3 min (×1 cycle), and then 35 cycles of 30 s at 94°C , 30 s at 55°C and 30 s at 72°C, followed by a final extension of 7 min at 72°C. All amplifications were performed in volumes of 25 μ1 containing 10 mM Tris-HCl (pH 9), 50 mM KCl, 3 mM $MgCl_2$, 250 μM dNTPs 1.5 μM each primer, 100 ng of genomic DNA, and 2.5 U of Taq polymerase. PCR products were electrophoresed (30–60 min at 90 volts) in 1% agarose gel in Tris-acetate-EDTA buffer, pH 8. Gels were stained with ethidum bromide (1.5 μg) and viewed with a UV transilluminator.

CVYV RT-PCR

One hundred and thirty-three cucurbit samples showing CVYV symptoms (vein-clearing and leaf chlorosis on older leaves), were collected from eastern and central regions of Iran. Total RNA extracts (TRI-Reagent, Sigma Chemical) were obtained and RT-PCR was carried out using CVYV-specific primers designed according to the partial sequence of the coat protein gene of CVYV-Isr (AF233429) (Lecoq *et al.,* 2000) (Table 2).

Results

Identification and distribution of viruses in cucurbit samples

We analyzed 1699 samples taken from 43 melon, 36 squash, 22 watermelon, and 22 cucumber commercial fields in 17 provinces of Iran. During the surveys (2005 and 2006), CABYV, WMV, ZYMV, CMV, and WmCSV were identified in all four cucurbit species. CABYV, WMV, and ZYMV were detected in all the provinces. CMV, MNSV, PRSV-W, SqMV, CGMMV, ZYFV, and WmCSV were detected in 15, 4, 9, 7, 5, 4, and 3 of the 17 provinces respectively (Fig. 1 and Table 3). We failed to detect SqMV, CGMMV, MNSV, PRSV, and ZYFV in watermelon, CVYV in squash, MNSV, and ZYFV in cucumber. CABYV, ZYMV, WMV, and CMV were also detected in asymptomatic samples.

Samples that were positive (ZYMV and CABYV) using DAS-ELISA were checked by RT-PCR to con-

Table 2. Primers used in RT-PCR and predicted amplicon size for the detection of ZYMV, CABYV and CVYV.

Primer	Sequence	Amplicon size (bp)
ZYMV-CP-5´	5´-GGTTCATGTCCCACCAAGC-3´	600
ZYMV-CP-3´	5´-ATGTCGAGTATCACATTTCC-3´	
CABYV-CP-5´	5´-CGCGTGGTTGTGGTCAACCC-3´	479
CABYV-CP-3´	5´-CCYGCAACCGAGGAAGATCC-3´	
CVYV-CP-5´	5´-GCTTCTGGTTCTCAAGTGGA-3´	540
CVYV-CP-3´	5´-GATGCATCAGTTGTCAGATG-3´	

Table 3. Distribution of viruses in the 17 provinces of Iran surveyed in this study.

Year/Province	Virus detected										
	CABYV	WMV	ZYMV	CMV	SqMV	CGMMV	WmCSV	MNSV	PRSV	ZYFV	CVYV
2005											
Khorasan	M,W,S,C[a]	M,W,S	M,W,S,C	M,W,S,C	-[b]	S,C	-	-	-	-	-
Semnan	M,S	M	M,S	M,W,S,C	M	M,S,C	-	-	M	-	n.d.
Tehran	M,S,C	M,W,S	M,W,S	M,W,S,C	-	S,C	-	-	S	-	-
Esfahan	M,S,C	M,W,S,C	M,W,S,C	C	S	S,C	-	-	-	-	-
2006											
Azarbaijan-E-Sharghi	S,C	S,C	S,C	S,C	-	-	-	-	-	-	n.d
Azarbaijan-E-Gharbi	S,C	M,S,C	M,S,C	M,S,C	-	-	-	-	-	-	n.d
Kordestan	S	S,C	S,C	S	-	-	-	M,S	-	-	n.d
Hamedan	M,S	S	M,S	M,S	S	-	-	M,S	M,S	S	n.d
Markazi	M,S	M,S,C	M,S,C	M,S,C	-	-	-	-	M,S	-	
Yazd	S	S,C	S,C	S,C	S	-	-	M	S	-	n.d
Fars	M,S,C	M,W,S,C	M,S,C	M,S,C	-	-	-	-	-	-	-
Kerman	S	W,S,C	S	M,S,C	-	-	M,W,S,C	-	-	-	M,W,C
Hormozgan	C	W,C	C	-	-	-	M,W,S,C	-	-	S	n.d
Sistan-Baluchestan	M,C	M	M	M	M	-	-	-	M	M	
Khozestan	W,S,C	M,W,S,C	M,W,S,C	-	S	-	-	-	S	-	n.d
Mazandaran	S,C	S,C	S,C	M,C	S,C	-	-	M,S	M,C	S	n.d
Golestan	M,W,S	M,W,S	W,S	S	-	S,C	S,C	-	S	-	n.d

[a], Positive reaction: M, melon; S, squash; C, cucumber; W, watermelon.
[b] -, Negative reaction.
n.d., The samples were not checked for that virus.

firm ZYMV and CABYV identification. One DNA amplification product of approximately 479 bp or of 600 bp was observed in samples that were positive to CABYV or ZYMV respectively in DAS-ELISA. No DNA product from healthy plant extracts was amplified. RT-PCR results confirmed that ZYMV and CABYV occurred on all four cucurbit species and in all provinces surveyed.

CVYV was identified by a differential host range reaction and RT-PCR. Typical vein clearing symptoms were seen following mechanical inoculation of cucumber and melon plantlets, but no symptoms were seen in *Chenopodium amaranticolor* and *C. quinoa*. A 540 bp amplicon corresponding to the central region of the CVYV coat protein was obtained from extracts of watermelon, melon and cucumber samples collected from Kerman province, but not from healthy plant extracts. CVYV was not detected using RT-PCR in 123 additional symptomatic cucurbit samples collected from Fars, Esfahan, Tehran, and Khorasan provinces.

Incidence of cucurbit viruses

During the two-year survey, almost 71% of tested plants were infected by at least one of the viruses. In 2005, laboratory testing of 428 collected cucurbit samples indicated that the incidence of viral infection overall was 84% (Table 4), with an incidence of infection in squash, cucumber, melon, and watermelon samples of 88, 84, 83, and 77% respectively. In 2006, laboratory testing of the 1271 collected cucurbit samples indicated that the incidence of viral infection overall was 66%. The incidence of infection in squash, watermelon, cucumber, and melon samples was 85, 75, 58, and 49% respectively (Table 4). The incidence of the viruses infecting melon, squash, watermelon and cucumber in 2005 and 2006 is shown in Table 4. CABYV was by far the most common virus, infecting about 49, 47, 40, and 33% of the cucumber, squash, melon and watermelon samples respectively. CABYV was followed in frequency by WMV, ZYMV, and CMV, which infected about 28, 26, and 13% of the cucurbit samples respectively (Table 5). In cucumber, CMV and ZYMV were the most common (33%), followed by CABYV (21%), while in squash, ZYMV and WMV were the most common, with an incidence of 38 and 26% respectively. In melon and watermelon samples, WMV and ZYMV were the most common, followed by CABYV, with an incidence of 33, 30 and 21% respectively (Table 5). The viruses SqMV, CGMMV, WmCSV, MNSV, PRSV and ZYFV were detected in less than 2% of all samples. During the surveys, the proportion of plants with yellowing symptoms was visually estimated for each field. These observations, together with the analyses of virus detection in the samples, provided an estimate of CABYV incidence in the fields surveyed. In 63% of the fields, the incidence of CABYV was greater than 20%.

Mixed infections

Almost half of the infected samples (49%) showed mixed infections with two, three, or more viruses (Table 6). In 2005, approximately 69% of symptomatic samples were infected with two or more viruses, compared with about 41% in 2006. The proportion of mixed infections in squash, melon, cucumber, and watermelon was 53, 63, 38, and 19% of symptomatic samples respectively (Table 6). In the two years of the survey, WMV+CABYV was the most frequent double infection in melon and WMV+ZYMV in watermelon (Table 6). With squash and cucum-

Table 4. The total number of cucurbit samples infected by at least one virus/total number of samples in 2005 and 2006.

Crop	No. infected/total No. of samples (%)		
	2005	2006	Total
Melon	147/177 (83)	231/469 (49)	378/646 (59)
Squash	103/117 (88)	360/424 (85)	463/541 (86)
Watermelon	37/48 (77)	131/175 (75)	168/223 (75)
Cucumber	72/86 (84)	118/203 (58)	190/289 (66)
Total	359/428 (84)	840/1271 (66)	1199/1699 (71)

Table 5. Number of plants infected by the following viruses: CABYV, WMV, ZYMV, CMV, SqMV, PRSV-W, WmCSV, CGMMV, MNSV and ZYFV as determined by DAS-ELISA.

Crop	No. samples tested	No. of infected samples									
		CABYV	WMV	ZYMV	CMV	SqMV	CGMMV	WmCSV	MNSV	PRSV	ZYFV
Melon	646	258	211	135	62	12	24	4	16	17	4
Squash	541	254	138	205	53	18	4	7	5	14	5
Watermelon	223	74	67	46	7	2	0	8	0	2	0
Cucumber	289	141	55	61	95	2	0	3	2	2	0
Total (%)	1699	727 (43)	471 (28)	447 (26)	217 (13)	34 (2)	28 (2)	22 (1)	23 (1)	35 (2)	9 (0.5)

ber, WMV+CABYV was the most frequent double infection in 2005, and ZYMV+CABYV in 2006 (Table 6), followed by WMV+ZYMV in squash, and WMV+CABYV in cucumber, in 2006. The most common triple infection in squash, melon, cucumber and watermelon was ZYMV+WMV+CABYV. The most frequent quadruple infection in squash, melon, and cucumber was ZYMV+WMV+CABYV+CMV.

Discussion

Cucurbit viruses have always caused major losses in the quantity and quality of cucurbit crops worldwide and they represent one of the most important limiting factors for growers (Provvidenti, 1996). In Iran, cucurbits have a high incidence of symptoms suggestive of viral infection. Despite the importance of cucurbits in Iran, in previous studies only a limited number of samples from a few areas were tested for viruses, but their distribution and incidence in the major cucurbit cultivation regions were not determined. This is the first report of an extensive survey using serological and molecular diagnostic procedures to identify the most important viruses of cucurbit crops and determine their incidence.

In the two years of the survey, symptoms such as mosaic, yellowing, vein clearing and fruit deformation were found in all cucurbit fields surveyed with an incidence of between 20 and 80% of plants. Of the 1699 samples tested, 71% were infected by at least one virus. A significant percentage (29%) of samples with symptoms suggestive of virus infection did not react with any of the antisera used in the study, suggesting that other viruses were involved that still need to be identified.

Although ZYMV is one of the most important viruses infecting cucurbits worldwide and was first reported almost 18 years ago in Iran (Ghorbani, 1988), the data presented revealed that CABYV was the most common and widespread virus in open field crops of the provinces surveyed. Extensive surveys have shown that CABYV is one of the most common viruses in open-field crops in many regions having a variety of ecologies (Lecoq, 1999; Lecoq *et al.,* 2003). The high incidence of mosaic and leaf deformation tended to mask the yellowing symptoms induced by CABYV. This might explain why the infection went unnoticed for several years in Iran, and why attention has been directed to it only recently (Bananej *et al.,* 2006a).

The most common viruses in melon, squash, watermelon and cucumber were CABYV, WMV, ZYMV, and CMV. The survey indicated that CABYV, WMV, and ZYMV were widely distributed in the four cucurbit species in all areas surveyed. In agreement with our findings, field-grown cucurbit crops from Lebanon also show a high incidence of CABYV. The prevalence and distribution of CMV, ZYMV and CABYV as found in a recent survey in Lebanon showed that CABYV was the most common virus in zucchini and melon, whereas CMV was rather rare in those cucurbits (Abou-Jawdah *et al.,* 2000). In Greece, high incidences of cucurbit-infecting viruses have also been reported, including WMV (67%), ZYMV (27%), CMV (20%), CABYV (20%), PRSVW (3%) and SqMV (2%) (Papvassiliou *et al.,* 2002). WMV and CMV appeared to be the most widespread viruses in cucumber and zucchini samples in Bulgaria (Kostova *et al*., 2003).

In this survey, a number of viruses were detected in Iran for the first time: they were ZYFV in melon and MNSV in squash. MNSV, transmitted by the

Table 6. Multiple infections in squash, melon, cucumber, and watermelon during 2005 and 2006.

Crop	2005						2006						Total
	Double infected		Triple infected		More than triple infected		Double infected		Triple infected		More than triple infected		
	A[a]	Virus	A	Virus	A	Virus	A	Virus	A	Virus	A	Virus	A
Squash	43/103	WMV-CABYV WMV-ZYMV	29/103	ZYMV-WMV-CABYV 0	11/103	ZYMV-WMV-CMV-CABYV ZYMV-WMV-CMV-CABYV-CGMMV	108/360	ZYMV-CABYV WMV-ZYMV	42/360	ZYMV-WMV-CABYV ZYMV-CMV-CABYV	11/360	ZYMV-WMV-CMV-CABYV ZYMV-PRSV-CMV-CABYV	244/463
Melon	65/147	WMV-CABYV WMV-ZYMV	29/147	ZYMV-WMV-CABYV ZYMV-WMV-CMV	9/147	ZYMV-WMV-CMV-CABYV ZYMV-PRSV-CMV-CABYV	99/231	WMV-CABYV WMV-ZYMV	31/231	ZYMV-WMV-CABYV ZYMV-WMV-CMV	5/231	ZYMV-WMV-CMV-CABYV ZYMV-PRSV-CMV-CABYV	238/378
Cucumber	15/27	WMV-CABYV WMV-ZYMV	5/72	ZYMV-WMV-CABYV ZYMV-CMV-CABYV	21/72	ZYMV-WMV-CMV-CABYV ZYMV-WMV-WmC-SV-CA-BYV	18/118	ZYMV-CABYV WMV-CABYV	14/118	ZYMV-WMV-CABYV ZYMV-CMV-CABYV	0/118	0 0	73/190
Watermelon	14/37	WMV-ZYMV WMV-CABYV	5/37	ZYMV-WMV-CABYV ZYMV-CMV-CABYV	0/37	0 0	8/131	WMV-ZYMV WMV-CABYV	5/131	ZYMV-WMV-CABYV 0	0/131	0 0	32/168
Total	137/359		68/359		41/359		233/840		92/840		16/840		587/1199
Total per year			246/359 (69%)						341/840 (41%)				(49%)

[a] Multiple infected samples/ Total infected samples

soil fungus *Olpidium bornovanus* (Campbell *et al.,* 1995), was found only in 23 samples of melon and squash from Hamedan, Yazd, Kurdestan, and Mazandaran provinces (Fig. 1 and Table 3). However, MNSV is seed-borne (Campbell *et al.,* 1996) and could become more severe in the future.

WmCSV has only been reported from a few provinces in southern Iran (Bananej *et al.,* 1998; Kheyr-Pour *et al.*, 2000; Bananej *et al.*, 2002). The survey showed that WmCSV has spread to new hosts in Kerman province and has also spread to a new province (Golestan) in the north of Iran. This is the first report of WmCSV in a new host and a new region of the country. The survey also showed that CVYV occurred in the southern province of Kerman. CVYV (genus *Ipomovirus*, family *Potyviridae*) was first described in Israel (Cohen and Nitzany, 1960) and is now widespread in cucurbit crops in the Middle East and Mediterranean regions (Lecoq *et al.,* 2000). WmCSV and CVYV are transmitted by *Bemisia tabaci,* and since these vectors are found in most regions of Iran, the viruses may become a serious threat for cucurbit crops in future. Further surveys in areas where *B. tabaci* is common are required to get a better idea of the incidence of these whitefly-transmitted viruses in cucurbit crops in Iran.

Mixed infections were found in approximately 49% of infected samples. CABYV occurred in mixed infections in all cucurbits except watermelon. In 1998 in Lebanon, some 13% of melon and 30% of squash plants showed mixed infections of ZYMV+CABYV (Abou-Jawdah et al., 2000). The highest incidence (15%) was found in cucurbits of Turkey infected with ZYMV+WMV (Sevik and Arli-Sokmen, 2003). Mixed infections are associated with enhanced symptom expression and synergistic effects are reported for viruses in the family *Luteoviridae* (Savenkov et al., 2001). The high incidence of CABYV found in the study, together with the high number of mixed infections, suggests that CABYV is an important threat for cucurbit crops in Iran.

This survey provides essential basic information useful for cucurbit virus control strategies in Iran. It revealed that aphid-borne CABYV, WMV, and ZYMV are the most common viruses causing severe symptoms in open fields. The widespread occurrence of viruses showed that the Iranian local varieties are very susceptible. The avoidance of aphid vectors is of fundamental importance in controlling these viruses. Several reports examine the use of plastic mulches to prevent or delay the onset of aphid-borne virus infections. Yield of field-grown squash in Lebanon is greatly affected by ZYMV and CABYV. A few studies in France (Lecoq et al., 1991), Taiwan (Wang et al., 1991) and the UK (Walkey et al., 1992) report on the successful use of a mild strain of ZYMV as cross-protection. In France, Lecoq et al. (1991) reported that this mild strain of ZYMV increased yield to 14 times that of the unprotected controls, under very high disease pressure. An important option for disease control would be the use of cultivars with genetic resistance to the virus. For example, several resistance sources for CABYV in melon have been described (Dogimont *et al.*, 1996, 1997). Evaluation of Iranian cucurbit germplasm for disease resistance as well as for other traits such as fruit quality could prove very promising.

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