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Comparative Biological and Molecular Variability of *Zucchini yellow mosaic virus* in Iran*

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Abstract: Strains of *Zucchini yellow mosaic virus* (ZYMV) were obtained from different cucurbit crops in different parts of Iran such as Isfahan (Is), Mazandaran (Sh), Karaj (Kr), Varamin (Vr), Khorasan (Kho), Kerman (Jr), Khuzestan (Dez and Jaz), Hamedan (Amz), Saveh (Sa), Markazi (Mkh), with one seed borne strain from Hamedan (Sdh) were partially characterized and compared. Variability was detected among strains regarding symptomatology and host range. These strains were different and distinguishable in the ability to infect specific hosts and divided into three groups. In one group, there were strains of Mazandaran, Karaj and seed borne Hamedan strain. In the second group, there were those of Varamin, Khorasan, Kerman, Isfahan, Saveh and Markazi province and in the third group there were strains of Khuzestan. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) of the NIb and N-terminal part of the coat protein coding region, followed by Restriction Fragment Length Polymorphism (RFLP) analysis of the PCR product of the thirteen mentioned strains with two restriction enzymes has been shown not to be an effective procedure for discriminating strains.

Key words: *Zucchini yellow mosaic virus*, host range, symptomatology, variability, RFLP

INTRODUCTION

Zucchini yellow mosaic virus (ZYMV), first described in Italy in 1973 (Lisa *et al*., 1981), is responsible for major economic losses in cucurbit crops in many parts of the world (Desbiez *et al*., 2002; Desbiez and Lecoq, 1997; Glasa and Pittnerova, 2006).

ZYMV belongs to the potyviruses, a group of plant viruses characterized by a monopartiate, positive-sense, single-stranded RNA genome encapsidated in flexuous, filamentous particles. The RNA is translated into a single polyprotein cleaved by three viral proteases. The 36 kDa coat protein of ZYMV encapsidates the viral RNA and is also involved in aphid transmissibility of the virus (Riechmann *et al*., 1992). Strains of ZYMV from distinct geographic origins exhibit biological diversity, particularly concerning their host range, symptomatology and aphid transmission (Desbiez *et al*., 1996, 2002).

Determining variability within a virus group and understanding mechanisms and factors affecting this variability are of considerable agronomic significance, particularly for determining resistance gene deployment strategies, since natural resistance genes can be rapidly overcome by adapted virus strains. In addition, variability of virus strain, particularly within the capsid protein, raises a problem for the development of reliable diagnosis techniques based on the antigenic properties of the coat protein.

In Iran, reports were arranged from 1968 to 2007, respectively to infect cucurbits: *Cucumber mosaic virus* (Manochehri, 1968), *Watermelon mosaic virus* (Weidemann and Mostafawy, 1972), *Cucumber green mottle mosaic virus* (Ghorbani, 1986), *Squash mosaic virus* (Izadpanah, 1987), *Zucchini yellow mosaic virus* (Ghorbani, 1988), *Watermelon chlorotic stunt virus* (Bananej *et al*., 1998),

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Cucurbit yellow stunting disorder virus (Keshavarz and Izadpanah, 2004), *Cucurbit aphid-borne yellows virus* (Bananej *et al*., 2006), *Melon necrotic spot virus* (Safaeezadeh, 2007) and *Zucchini yellow fleck virus* (Safaeezadeh, 2007). More recently, severe epidemics of ZYMV were observed (Ghorbani, 1988; Hosseinifarhangi *et al*., 2004; Massumi *et al*., 2007). In this study, biological and molecular methods were used to characterize the variability of ZYMV in this ecosystem in order to determine potential control strategies against this very destructive virus.

MATERIALS AND METHODS

Sample Collection

A survey was conducted from August 2005 to October 2006, in commercial fields of Iran from Khorasan to Khuzestan. There were collected 279 samples with virus like symptoms from leaves and occasionally from fruits of cucurbits. They were tested by double antibody sandwich ELISA (DAS-ELISA, see later) to detect the presence of mixed infections with other cucurbit viruses: ZYMV, WMV, PRSV, CMV, SqMV, MNSV, WmCSV, ZYFV and CABYV. Finally, 13 samples showing the highest absorbance values in ELISA to ZYMV antisera were used for this study (Table 1).

To ensure the purity of each strain, all strains (except the strains from Khuzestan) were passed through three successive single-lesion transfers on *Chenopodium amaranticolor* Coste and Reyn. Each strain was maintained separately in Peto seed zucchini squash (*Cucurbita pepo* L.), which also served as the source of inoculum.

Host Range

At least 43 plants of each species or cultivar belonging to the families Amaranthaceae, Asteraceae, Brassicaceae, Chenopodiaceae, Cucurbitaceace, Euphorbiaceae, Fabaceae, Liliaceae, Linaceae, Malvaceae, Poaceae, Solanaceae and Umbeliferae were inoculated by rubbing inocula on leaves previously dusted with 400-mesh carburandum. Incula derived from freshly harvested leaves of greenhouse grown infected zucchini plants ground in 1% M potassium phosphate buffer, pH 7.2. The plants were maintained in a greenhouse at 25-30°C. Virus symptoms on plants were recorded 2 weeks after inoculation and then at regular intervals during the next 4 weeks. All plants showing no symptoms were assayed for virus infections by back inoculation of indicator hosts or by ELISA (Clark and Adams, 1977).

ELISA

DAS-ELISA with polyclonal antisera was used to check the presence of ZYMV and the absence of other cucurbit viruses: WMV, PRSV, CMV, SqMV, MNSV, WmCSV, ZYFV and CABYV. The double antibody sandwich (DAS-ELISA) (Clark and Adams, 1977) method was performed and all buffers were prepared according to the manufacturer's instruction (Loewe, Biochemica Gmbh, Sauerlach, Germany and INRA, France). Cucurbit leaf samples were ground in sterile mortar and pestle with the extraction buffer (PBST: 0.13 M NaCl, 0.014 M KH_2PO_4, 0.08 M Na_2HPO_4, 0.002 M KCl, pH 7.4) containing 0.05% Tween 20 and 0.1% non-fat dry milk and added to wells, which had been precoated with ZYMV, WMV, PRSV, CMV, SqMV, MNSV, WmCSV, ZYFV and CABYV specific polyclonal antisera (Loewe, Biochemica Gmbh, Sauerlach, Germany and INRA, France) was diluted in carbonate buffer (pH 9.6). Plates (Nunc Microwell, Roskilde, Denmark) were incubated at 4°C overnight and washed four times with PBST-Tween 20 buffer. Then, plates were 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 μg mL^{-1}, pH 9.8) was added to each well and incubated at room temperature for 30 to 120 min. Absorbance values were read

Table 1: Origins of ZYMV strains from Iran and their reactions with 9 Antisera at DAS-ELISA test

ZYMV strain	Location	Host plant	ZYMV	CMV	WMV	PRSV-W	MNSV	SqMV	ZYFV	WmCSV	CABYV
ZYMV-Is	Isfahan	Zucchini	+	+	+	+	-	-	-	-	-
ZYMV-Sh	Mazandaran	Cucumber	+	-	-	-	-	-	-	-	-
ZYMV-Kr	Karaj	Zucchini	+	+	-	-	-	-	-	-	-
ZYMV-Vr	Varamin	Cucumber	+	+	-	-	-	-	-	-	-
ZYMV-Ya	Yazd	Pumpkin	+	+	+	-	-	-	-	-	-
ZYMV-Kho	Khorasan	Melon	+	+	+	-	-	-	-	-	-
ZYMV-Jr	Kerman	Cucumber	+	-	+	-	-	-	-	-	-
ZYMV-Dez	Khuzestan-Dez	Pumpkin	+	-	-	-	-	-	-	-	-
ZYMV-Jaz	Khuzestan-Jazayer	Watermelon	+	-	-	-	-	-	-	-	-
ZYMV-Amz	Hamedan-Amzajerd	Cucumber	+	-	-	-	-	-	-	-	-
ZYMV-Sdh	Seed-Borne/ Hamedan strain	Pumpkin	+	-	-	-	-	-	-	-	-
ZYMV-Mkh	Markazi-Khushkrood	Snake cucumber	+	-	-	-	-	-	-	-	-
ZYMV-Sa	Saveh	Muskmelon	+	-	-	-	-	-	-	-	-

+: Positive, - : Negative

at 405 nm using a microplate reader. Virus-free cucurbit species grown in an insect-proof growth chamber were used as negative controls. Samples were considered to be positive when the absorbance at 405 nm (A_{405}) values exceeded the mean of the negative controls (healthy) by at least a factor of two (Al-Shanwan *et al*., 1995; Sammons *et al*., 1989). All samples were assayed in three repeats. The presence of ZYMV was confirmed by RT-PCR.

RNA Extraction

Total RNA was extracted from 50 mg ZYMV-infected leaves using TRI-Reagent (Molecular Research Center, Inc., Cincinnati, OH) according to the procedure of Lecoq *et al*. (2004) and resuspended in 50 mL RNase-free water. Healthy zucchini extracts were used as controls.

RT-PCR Amplification

In addition to serological detection, 13 samples (Table 1) used for biological variability and positive in ELISA were tested by RT-PCR technique. In this procedure, oligonucleotide primers (Reverse primer: 5'-ATGTCGAGTATCACATTTCC-3': 8200-8220 and forward primer 5'-GGTTCATGTCCCACCAAGC-3': 8800-8819) were designed to amplify a fragment of the NIb and CP coding regions of ZYMV (about 600 bp), overlapping the variable N-terminal part of the CP (Lecoq *et al*., 2004). These primers were synthesized by MWG-Biotech. Co. (Germany).

RT-PCR was performed in a two-step format using the extracted total RNA. Reverse transcription reaction was done in 25 μL volumes containing 4 μL of template RNA, 1 μL of the reverse primer RT (100 pmol μL^{-1}) and 1 μL of RevertAid™ M-MuLV reverse transcriptase (Fermntas, Lithuania). This reaction was carried out using a top-heating thermal cycler at 42°C for 60 min and stopped by incubation at 70°C for 10 min, as suggested by the manufacturer. For PCR amplification in 25 reaction volumes, 1 μL of the primers forward and reverse (100 pmol μL^{-1}), 2.5 μL of 10X *Taq* reaction buffer (200 mM Tris-HCl, 500 mM KCl, pH 8.4), 0.75 μL $MgCl_2$ (50 mM), 0.5 dNTPs (10 mM) and 2.5 units *Taq* DNA polymerase (CinnaGen Inc., Tehran, Iran) were added to each 5 μL of first-strand cDNA reaction mixture. The PCR program consisted of a 3 min heating step at 94°C, followed by 35 cycles of amplification step of 30 sec at 94°C, 30 sec at 55°C and 30 sec at 72°C. Then, 7 min at 72°C was performed.

PCR products and DNA ladder (GeneRuler™ 250 bp DNA Ladder Plus, Fermntas, Lithuania) were analyzed by electrophoresis through 1% agarose gels in the presence of 1 μg mL^{-1} ethidium bromide using 1 X Tris-Borate EDTA (TBE) buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2EDTA, pH 8.3) (Sambrook *et al*., 1989). Gels were visualized and photographed with UV-illuminator.

After electrophoresis, the amplified DNA fragments were purified with DNA purification kit (Roche, Germany) according to manufacturer's procedure.

PCR/RFLP Analysis

For Restriction Fragment Length Polymorphism (RFLP) analysis, of the 600 bp fragment RT-PCR amplified products, there were used two restriction enzymes *Pvu*II and *Eco*RV (Roche, Germany) suggested by Lecoq *et al*. (2004) for grouping ZYMV strains. As well, preliminary study using webcutter software (Webcutter 2.0, copyright 1997 Max Heiman) confirmed that based on sequence data of the NIb and CP coding regions of ZYMV available in the Genbank, *Pvu*II and *Eco*RV restriction enzymes are very suitable for differentiation of ZYMV strains.

Aliquots (11 μL) or the PCR reactions were incubated with 1 μL restriction enzymes *Pvu*II and *Eco*RV, then buffer was added according to the manufacturer's instruction (Roche, Germany), so that the final volumes of 25 μL were incubated for 3 h at 37°C; the products were, then, analysed by electrophoresis in an agarose gel 2%, stained and photographed as above.

RESULTS

Host Range

Forty three plant species belonging to thirteen different families were tested. From the data shown in Table 2, it is evident that thirteen strains varied in host reactions. These strains were different and distinguishable in the ability to infect specific hosts and divided into three groups. In one group, there were strains of Mazandaran, Karaj and seed borne Hamedan strain. In the second group, there were those of Varamin, Khorasan, Kerman, Isfahan, Saveh and Markazi province and in the third group there were strains of Khuzestan.

Mazandaran, Karaj and seed borne Hamedan strain were able to infect *Phaseolus vulgaris* L. cv. Rashti (Fig. 1G), *Pisum sativum* L. cv. Denmarki and *Vigna unguiculata* (L.) Walp. cv. Poloe while other strains were not (Table 2).

Although there were similarities between mentioned strains and divided into three groups, there was some variability among them. ZYMV-Kr differed from other strains by inducing vein-banding infection in cucurbits and infected *Phaseolus vulgaris* L. cv. Rashti, *Pisum sativum* L. cv. Denmarki and *Vigna unguiculata* (L.) Walp. cv. Poloe. ZYMV-Is induced severe shoe-string in every cultivar of *Cucurbita pepo* L. ZYMV-Vr, ZYMV-Kho, ZYMV-Dez, ZYMV-Ya and ZYMV-Sdh induced necrotic local lesions on inoculated leaves in *Cucurbita pepo* L., whereas ZYMV-Sh and ZYMV-Kr never induced yellowing in their natural hosts (Table 2).

Failure to recover the virus by back inoculation to *C. pepo* L. indicated that studied ZYMV strains could not infect noncucurbit plant species including *Allium cepa* L.; *Amaranthus retroflexus* L.; *Apium graveolens* L.; *Beta vulgaris* L.; *Brassica oleracea* L.; *Capsicum annuum* L.; *Cicer arietinum* L.; *Datura inoxica* Mill.; *Gomphrena globosa* L.; *Hibiscus esculentus* (L.); *Lactuca sativa* L.; *Lathyrus odoratus* L.; *Lens culinaris* Medik.; *Lepidium sativum* L.; *Linum usitatissimum* L.; *Solanum lycopersicum* L.; *Malva neglecta* Wallr.; *Melilotus officinalis* (L.) Lam.; *Nicotiana rustica* L.; *Nicotiana tabacum* L.; *Petunia axillaris* (Lam.); *Raphanus sativus* L.; *Ricinus communis* L.; *Sinapis alba* L.; *Solanum melongena* L.; *Solanum nigrum* L.; *Trigonella foenum-graecum* L.; *Triticum aestivum* L. and *Vicia hirsuta* (L.). Similar results were observed when inoculated leaves of these plant species were checked by DAS-ELISA.

Table 2: Host reaction thirteen strains[a] of *Zucchini yellow mosaic virus* (ZYMV) in different hosts

	Symptoms[b] of ZYMV strains												
Test plants	Is IL/UL	Sh IL/UL	Kr IL/UL	Vr IL/UL	Ya IL/UL	Kho IL/UL	Jr IL/UL	Mkh IL/UL	Sa IL/UL	Jz IL/UL	Dez IL/UL	Amz IL/UL	Sdh IL/UL
Cucurbitaceae													
Cucurbita pepo Peto seed	sl/sym, b,ld,sh	sl/sym, b,ld	sl/vb, b	nll/sy, b,ld,sh	nll/sy, mld,n	nll/sy, d,b,ld	sl/sym, b,d, ssh	sl/sm, vb,b,ld	nll/sy, b,ld,sh	nll/sm, ld	sl/sm, ld	sl/sm, vb,b,ld	nll/sym,b,n
C. pepo Takata	sl/sym, ld,sh	sl/sm	sl/sm	nll/sym	nll/sym, sh	nll/sym, ld	sl/sym, b	sl/sm, sh,b,vb	nll/sym	nll/sym, sb,ssh	sl/sym, s,b,ssh	sl/sm,sh, b,vb	nll/sym,ld
C. pepo Hamedan	sl/sym, b,ld,sh	sl/sym, b,ld	sl/vb, b	nll/sy, b,ld,sh	nll/sym, mld	nll/sy, d,b,ld	sl/sym, d,s,sh	sl/sm, vb,b,ld	nll/sy, b,ld,sh	nll/sm, sb	sl/sm,sb	sl/sm, vb,b,ld	sl/sym, ld
Cucumis sativus Harst	sl/sym, b,ld	sl/sm, b,ld,d	sl/md	sl/sm, b,sd,sh	sl/sm,b, sd,sh	sl/sm, sb	sm/sl, b,ld	sl/sm, b,ld	sl/sm, b,sd,sh	sl/sm, ld,b	sl/sm, ld,b	sl/sm, b,ld	sl/sym, b,ld
C. sativus Mahalli	sl/sym, b,ld	sl/sm b,ld,d	sl/mm, d	sl/sm, b,d	sl/sm,b, sd,sh	sl/sm, sb	sm/sl, b,ld	sl/sm, b	sl/sm, b,d	sl/sm, ld,b	sl/sm, ld,b	sl/sm,b	sl/sym,b,ld
C. melo Mashhad	sl/sy	sl/sy	sl/sy	sl/sy,b	sl/sy	sl/sy,b	sl/sy	sl/sy	sl/sy,b	sl/sym	sl/sym	sl/sy	sl/sy
C. melo Hamedan	sl/sy	sl/sy	sl/sy	sl/sy, ld	sl/sy,ld	sl/sy,b	sl/sy	sl/sy	sl/sy, ld	sl/sy	sl/sy	sl/sy	sl/sy
Citrullus lanatus Harst	sl/d	sl/d	sl/d	sl/d	sl/m,d	sl/d	sl/sm,d	sl/d	sl/d	sl/d	sl/d	sl/d	sl/d
Luffa acutangula	sl/sm	sl/mm	sl/mm	sl/sm	sl/sm	sl/mm	sl/m	sl/sy	sl/sm	sl/sl	sl/sl	sl/sy	sl/sy
C. sativus var. *flexuosus*	sl/sy	sl/sm	sl/sm	sl/sy	sl/sym	sl/sy	sl/sm	sl/sym	sl/sy	sl/sm	sl/sm	sl/sym	sl/sym
Cucurbita moschata Hamedan	sl/sym, b,n	sl/sm, b	sl/b, vb	nll/sym, b,n	nll/sym, n	sl/sym, sb	sl/sym, sb	sl/sm, b	nll/sym, b,n	sl/sym	sl/sym	sl/sm,b	sl/sm,b
C. moschata PS	sl/sym, b,n	sl/sm,b	sl/sb, vb	nll/sym, b,n	nll/sym, b,d,n	nll/sym, b,d,n	sl/sym, sb	sl/sm,b	nll/sym, b, n	nll/sm, sb	sl/sm,sb	sl/sm,b	nll/sym,b,n
C. maxima Mahally	sl/sym, b,n	sl/sm, b	sl/sb, vb	nll/sym, b,n	nll/sym, b,n	nll/sym, b,n	sl/sym, sb	sl/sm,b	nll/sym, b,n	nll/sm, sb	sl/sm,sb	sl/sm,b	nl/sym
Fabaceae													
Vigna unguiculata Poloe	[c]-	sl/mm	sl/mm	-	-	-	-	-	-	-	-	-	sl/mm
Pisum sativum Denmarki	-	sl/sl	sl/sl	-	-	-	-	-	-	-	-	-	sl/sl
Pisum sativum Hamedan	-	sl/sl	sl/sl	-	-	-	-	-	-	-	-	-	sl/sl
Phaseolus vulgaris Rashti	-	sl/sm	sl/sm	-	-	-	-	-	-	-	-	-	sl/sm
Pedaliaceae													
Sesamum indicum	-	-	-	-	-	-	-	-	sl/sm	-	-	-	-
Chenopodiaceae													
Chenopodium amaranticolor	nll/-	red/-	red/-	nll/-	nll/-	nll/-	nll/-	nll/-	nll/-	-	-	nll/-	red/-
Chenopodium quinoa	nll/-	red/-	red/-	nll/-	nll/-	nll/-	nll/-	nll/-	nll/-	-	-	nll/-	red/-

[a]: Strains are designated as Is (Isfahan), Sh (Mazandaran), Kr (Karaj), Vr (Varamin), Ya (Yazd), Kho (Khorasan), Jr (Kerman), Mkh (Markazi-Khushkrood), Sa (Saveh), Jaz (Khuzestan-Jazayer), Dez (Khuzestan-Dez), Amz (Hamedan-Amzajerd), Sdh (Seed-borne Hamedan).

[b]: IL = Infected Leaves, UL = Upper Leaves; b = blister; d = dwarf; ld = leaf distortion; m = mosaic; mld = mild leaf distortion; mm = mild mosaic; n = necrosis; nll = necrotic local lesions; red = reddening spot; sb = severe blister; sh = shoe-string; sl = symptomless infection; sm = systemic mosaic; ssh = severe shoe-string; sy = systemic yellowing; sym = systemic yellow mosaic; vb = vein-banding. The reactions were confirmed by ELISA tests.

[c]: = No symptom and negative reaction in ELISA test

Symptoms induced by ZYMV strains in *Chenopodium amaranticolor* Coste and Reyn and *C. quinoa* Willd were considerable. ZYMV-Kr, ZYMV-Mkh and ZYMV-Sdh induced reddening spot (Fig. 1E); ZYMV-Vr, ZYMV-Kho, ZYMV-Is, ZYMV-Ya, ZYMV-Mkh, ZYMV-Sa, ZYMV-Jr and ZYMV-Amz induced necrotic local lesions (Fig. 1F), while ZYMV-Dez and ZYMV-Jaz did not induced any symptoms on these indicator plants.

RT-PCR and RFLP

Greenhouse plants with the highest values determined in ELISA were chosen to purify the putative ZYMV with one pair of primers. A PCR product with the expected size of approximately 600 bp was obtained for each of the RNA extracts investigated (Lecoq *et al*., 2004). No differences were observed among the sizes of PCR products of zucchini leaf extracts by using the same primers.

Of the restriction enzymes tested, *Eco*RV and *Pvu*II did not give distinguishable RFLP patterns with the PCR products from all the strains. The ZYMV strains could not be differentiated by digestion with *Eco*RV and *Pvu*II. There were not also distinctive polymorphism which could be resolved on polyacrylamide gels that could be generated with *Eco*RV and *Pvu*II (Fig. 2).

Fig. 1: Symptoms induced by ZYMV strains in Iran. (A) leaf deformation and shoe-string associated with natural infection by ZYMV on zucchini squash (*Cucurbita pepo* L. cv. Hamedan) in Khuzestan province; (B) shoe-string on zucchini squash (*Cucurbita pepo* L. cv. Hamedan) induced by ZYMV-Is, 20 days after mechanical inoculation; (C) leaf deformation and blister on cucumber (*Cucumis sativus* L. cv. Mahali) induced by ZYMV-Sh, 20 days after mechanical inoculation; (D) vein-banding in zucchini squash (*Cucurbita pepo* L. cv. Hamedan) induced by ZYMV-Sdh, 2 weeks after mechanical inoculation; (E) reddening spot on *Chenopodium amaranticolor* Coste and Reyn induced by ZYMV-Sdh, 2 weeks after mechanical inoculation; (F) necrotic local lesions on *Chenopodium quinoa* Willd induced by ZYMV-Sa, 2 weeks after mechanical inoculation; (G) mild mosaic on *Phaseolus vulgaris* L. cv. Rashti induced by ZYMV-Sdh, 2 weeks after mechanical inoculation

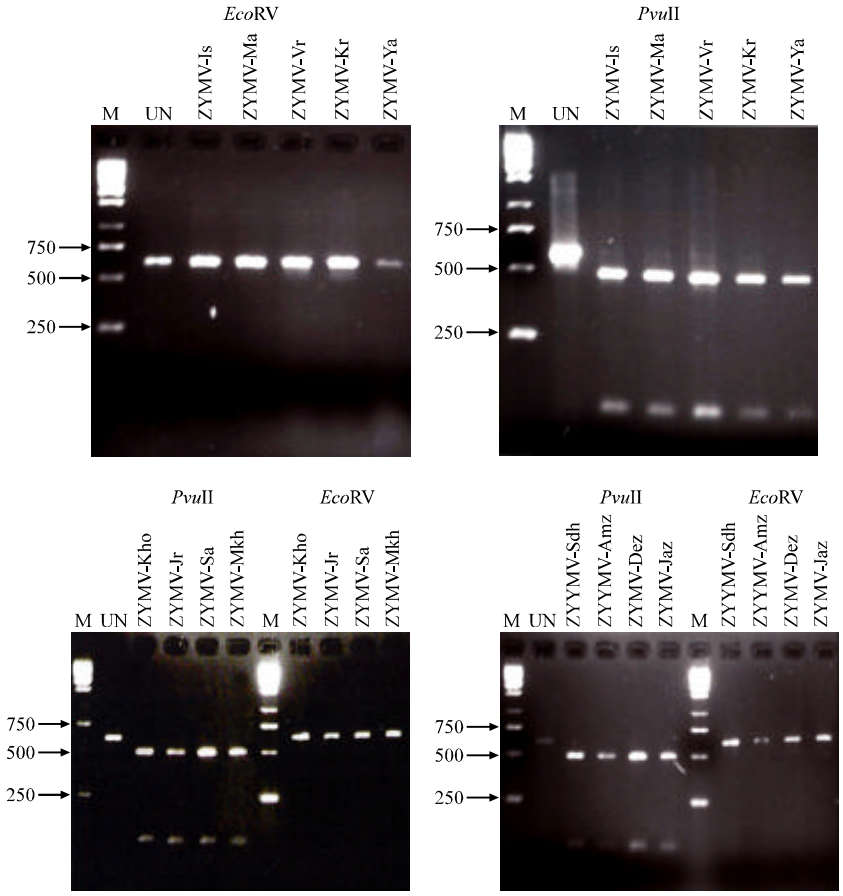

Fig. 2: Restriction Fragment Length Polymorphism (RFLP) of Reverse-Transcription Polymerase Chain Reaction products from strains of *Zucchini yellow mosaic virus* (ZYMV) with *Eco*RV and *Pvu*II. M, molecular weight marker; UN, undigested strain

DISCUSSION

ZYMV was shown to possess a range of variability in Iran and a significant biological variability was observed among thirteen strains of ZYMV from Iran collected from different hosts and locations within a 2 year period. In this study, variants were isolated from a limited geographical area soon after the first report of a ZYMV epidemic. Some diversity was observed in symptom expression: three strains induced vein-banding in cucumber and squash.

The fact that ZYMV-Kr, ZYMV-Sdh and ZYMV-Sh infected *Phaseolus vulgaris* L. cv. Rashti, *Pisum sativum* L. cv. Denmarki and *Vigna unguiculata* (L.) Walp. cv. Poloe but other did not (Table 2), suggests that deduced amino acid sequences of their HC-pro gene proteins are different and other regions in the ZYMV genome are also involved in causing different host responses. Establishment of systemic infection is a complex process, requiring a balance of the rates of replication, cell-to-cell movement and long-distance movement. In each of these phases there are interactions between virus proteins and host components (Suehiro *et al*., 2004). Recently a single amino acid change in the P3 gene was also shown to affect symptom severity of ZYMV in cucurbits plants (Desbiez *et al*., 2003).

In this study, host range results differed slightly from those of previous reports (Kwon *et al*., 2005; Mahgoub *et al*., 1998; Wang *et al*., 1992). Under certain conditions, thirteen strains of ZYMV can be distinguished by differences in symptomatology and host range. Although thirteen strains of ZYMV have distinctive entities, it is often difficult, under field conditions, for a researcher to differentiate among the symptoms caused by these strains. Therefore, other indicator hosts, *Phaseolus vulgaris* L. cv. Rashti, *Pisum sativum* L. cv. Denmarki and *Vigna unguiculata* (L.) Walp. cv. Poloe are useful for differentiation of strains of ZYMV.

The results of RT-PCR analyses using specific primers for ZYMV were in complete agreement with DAS-ELISA results.

Restriction profiles of ZYMV strains were not clearly different from each other and RFLP analysis of PCR fragments were not distinguished between strains of ZYMV. These strains did not contain *Eco*RV recognition site. The analysis of the amplified fragment by digestion by *Pvu*II revealed the presence of *Pvu*II recognition sites on every strain, collected from different parts of the country, but this site is similar in all of strains. Thus these restriction enzymes were not able to discriminate between ZYMV strains.

According to biological variability observed among studied strains, it is hypothesised that these restriction enzymes would be able to differentiate strains into three groups. Barbara *et al*. (1995) have shown that it is possible to discriminate among isolates of ZYMV by a simple PCR-RFLP procedure. A similar analysis of the CP gene using the frequent cutting restriction enzymes *Hpa*II and *Mse*I showed the distinctiveness of the Italian isolates, but failed to discriminate among other isolates. Although no sequence information is available for ZYMV isolates from UK and France as investigated by Barbara *et al*. (1995), it is possible to compare the reported RFLP patterns of these two isolates with the deduced RFLP patterns of sequenced isolates.

The limited molecular variability of ZYMV strains from Iran was neither correlated with their biological variability, nor with their geographical origin in the country.

The fact that *Pvu*II and *Eco*RV restriction enzymes which characterized French strains of ZYMV failed to characterize those from Iran suggested that Iranian strains of ZYMV greatly differed from those of France. Of course to confirm this point, it would be better to compare several strains of ZYMV collected from every province of Iran and then compare their biological and molecular variability with other studied strains of ZYMV. Finally, in order to determine Iranian ZYMV evolutionary relationships and study their genetic diversity, nucleotide sequencing of the NIb and N-terminal part of the coat protein coding regions are required to discriminate between Iranian strains of ZYMV with those previously deposited in the Genbank (Choi *et al*., 2007; Desbiez *et al*., 2002; Glasa and Pittnerova, 2006; Pfosser and Bauman, 2002).

The introduction of ZYMV to Iran or at least the appearance of epidemics must be recent, because no typical symptoms of ZYMV were noticed before 1988. The introduction of ZYMV to Iran may have occurred either through importation of infected plants or seeds, since ZYMV may be seed-transmissible in zucchini squash at a very low rate (Schrijnwerkers *et al*., 1991), or through migration of viruliferous aphids from neighboring countries where the virus is present i.e., Turkey (Davis and Yilmaz, 1984) or Pakistan (Ali *et al*., 2004; Desbiez and Lecoq, 1997). Long distance spread of potyviruses by viruliferous aphids has indeed been reported occasionally (Zeyen *et al*., 1987).

ZYMV is widespread in different cucurbit growing regions of Iran (Ghorbani, 1988; Hosseinifarhangi *et al*., 2004; Massumi *et al*., 2007). This virus is transmitted from plant to plant by mechanical inoculations, insect vectors and seeds (Schrijnwerkers *et al*., 1991). Seeds transmissions are considered the major and the most efficient means of dissemination of this virus.

The present study reports comparative biological and molecular variability of ZYMV in Iran for the first time. The information obtained in this study will be helpful to improve control strategies for such a destructive virus in Iran. However, further investigations onto biological and molecular properties of ZYMV strains from fields and greenhouse of other parts of Iran should be carried out in this respect.

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