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Partial Biological and Molecular Characterization of *Cauliflower mosaic virus* Isolates in Iran

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Abstract: The biological and molecular characteristics of nine *Cauliflower mosaic virus* (CaMV) isolates from infected cauliflower plants (*Brassica oleracea* var. *botrytis*), grown in different Iranian regions, were evaluated. Based on mechanical inoculation to a range of herbaceous indicators, the Iranian CaMV isolates were distinguished for their different capacity to infect cruciferous plants and *Datura stramonium*. Only a part of the isolates under study infected kohlrabi (*B. oleracea* var. *gongylodes*) and *D. stramonium* seedlings, whereas, in experimental trials, all of them were transmitted on healthy cauliflowers by green peach aphids (*Myzus persicae*). In PCR, 1.58 kb genomic fragments, corresponding to the CaMV ORF VI, were amplified. Their sequences showed close identities (96.1-96.7%) with that of D/H CaMV-Hungarian isolate. In phylogenetic analysis the isolates under study were clustered in a separate branch, together with the non-North American isolates group. The Iranian isolates were also well differentiated from other exotic CaMV isolates by restriction analysis using Hpy99I.

Key words: *Cauliflower mosaic virus*, Iranian isolates, host range, aphid transmission, ORF VI, sequencing

INTRODUCTION

CaMV, the type member of the genus *Caulimovirus*, is one of the most common and important viruses of brassica crops in the world. This virus is widespread in temperate regions wherever species of *Brassica* are grown (Sutic *et al*., 1999). CaMV has isometric particles about 50 nm in diameter, composed of 420 capsid protein subunits and a circular double-stranded DNA genome of 8 kbp (Cheng *et al*., 1992). The CaMV genome contains seven major ORFs (I to VII) and is replicated by the reverse transcription of an RNA intermediate (Mason *et al*., 1987; Haas *et al*., 2002). This virus induces a variety of systemic symptoms (chlorosis, mosaic, vein clearing and stunting) on many cruciferous plants, particularly on various *Brassica campestris* and *B. oleracea* cultivars, often in mixed infection with *Turnip mosaic virus* (TuMV) (Shepherd, 1981). All CaMV isolates can infect a wide variety of cruciferous species, but only a few of them, i.e., D4 and W260, can infect solanaceous species, including those of *Datura* and *Nicotiana* genera (Daubert *et al*., 1984; Daubert and Routh, 1990; Anderson *et al*., 1991; Qiu *et al*., 1997). CaMV is naturally transmitted by aphids in a non-circulative manner (Palacios *et al*., 2002), except for some isolates that are not at all transmitted (Lung and Pirone, 1973) and it uses the helper strategy for the transmission process (Pirone and Blanc, 1996; Gray and Banerjee, 1999). At least 27 aphid species are recognized vectors of CaMV (Kennedy *et al*., 1962). This virus is not transmissible by seed or pollen (Blanc *et al*., 2001). CaMV isolates have been previously characterized and differentiated on the basis of their reactions on various indicator plants, including turnip, kohlrabi and some solanaceous species (Lung and Pirone, 1972; Schoelz *et al*., 1986; Al-Kaff and Covey, 1995) and of the nucleotide sequence of their ORF VI gene. This ORF, in fact, is characterized by having high levels of variation if compared to the other CaMV ORFs and is therefore used to distinguish different isolates of the virus (Sanger *et al*., 1991; Chenault and Melcher, 1994a, b; Pique *et al*., 1995).

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In recent years, farm acreage of brassica crops, especially cauliflower, has significantly increased in Iran; the area covered by cauliflower passed from 800 ha with 20,000 tones production in 2000 to 1000 ha with 25,000 tones in 2003 (FAO, 2000, 2003). CaMV is a naturally occurring virus that infects cruciferous hosts in Iran (Shahraeen *et al*., 2003; Farzadfar *et al*., 2005, 2007), however, there is no information about the biological and molecular properties of Iranian isolates of the virus. In this study nine CaMV isolates from cauliflowers collected in different geographical areas of Iran were characterized using biological approaches. The molecular properties of these isolates were also studied by polymerase chain reaction (PCR) and sequencing of their ORF VI.

MATERIALS AND METHODS

Plant materials and serological assays: Cauliflower leaves with symptoms of deformation, mosaic, mottling, necrosis, rugosity, stunting, vein banding, vein clearing and/or yellowing were collected from 32 fields in Azarbayejan-e-gharbi, Esfahan, Khorasan, Fars, Tehran and Qazvin provinces of Iran during the summers of 2004 and 2005. These samples were tested for CaMV by double-antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977) using specific polyclonal antibodies purchased from Loewe (Sauerlach, Germany). In ELISA tests, all buffers were prepared according to the manufacturer's instructions. One hundred microliter of each leaf extract, ground in 1:5 (wt/vol) extraction buffer, were added to the wells of polystirene plates (Maxisorb, NUNC, Denmark) previously coated with 1:200 CaMV-IgG in carbonate buffer and incubated overnight at 4°C. The IgG-conjugated antibody incubation was done for 3 h at 35°C. Samples were considered positive if the absorbance at 405 nm, measured by using Multiscan-334 (Lab system, Finland), was greater than or equal to three times the healthy control mean values after 60 min of reaction with p-nitrophenyl phosphate at 1 mg mL^{-1} in substrate buffer.

Host range studies: Samples with positive ELISA readings were ground in 0.1 M Na-phosphate buffer (pH 7.4) containing 0.02% 2-mercaptoethanol and inoculated into radish (*Raphanus sativus*) dusted with carborundum. Virus isolates were purified by three serial single-lesion inoculation passages on turnip (*Brassica rapa*) or *Datura stramonium*. For subsequent experiments, nine CaMV isolates, different for provenience and severity of symptoms (Table 1), were propagated in radish. The infected leaf extracts (1:5 wt/vol) were rubbed onto a range of herbaceous plants shown in Table 2. In this study, the most common cruciferous weeds present in brassica fields, including *Eruca sativa* (rocket salad), *Hirschfeldia incana* (shortpod mustard), *Rapistrum rugosum* (rugose rapistrum), *Sisymbrium loeselii* (small tumble-mustard) and *S. irio* (London rocket) were also tested. Plants used in the host range studies were inoculated at four-six leaves stage, using at least three plants/indicator and kept under greenhouse conditions for 4-6 weeks; afterwards symptoms were recorded and virus presence determined by ELISA.

Aphid transmission: Aphid transmissibility of the selected CaMV isolates (Table 1) was performed with green peach aphids (*Myzus persicae* Sulzer). Aphids were starved for 1 hr, given a 5 min acquisition access on young leaves of *B. rapa* plants which had been previously infected (from 2-3 weeks) with the isolates under study and then moved on non-infected young turnips. Aphids were left to feed for 24 h and then killed by spraying with Confidor (Bayer, Germany). For each plant, 5 aphids were used and at least 10 test plants were assayed. The presence of CaMV in the inoculated plants was tested by ELISA.

DNA extraction and polymerase chain reaction (PCR): Upstream and downstream primers used in this study were, respectively, CaMV-6-F (5'-ACGCGTCGACATGGAGAACATAGAAAAAC-3') and CaMV-6-R (5'-ACGCGTCGACTCAATCCACTTGC TTTGAA-3'), designed by U. Melcher (Department of Biochemistry and Molecular Biology, Oklahoma State University). These primers, synthesized by MWG Biotech Co. (Ebersberg, Germany), direct the amplification of about 1,580 bp fragments containing the complete

Table 1: Iranian isolates of *Cauliflower mosaic virus* studied in this work

Isolate	Province	Symptoms	Accession No.
Ca-CAz22	Azarbayejan-e-gharbi	vein banding, mottling, leaf deformation	DQ870907
Ca-CAz26	Azarbayejan-e-gharbi	vein banding, mottling, leaf deformation	DQ870908
Ca-CE1	Esfahan	vein clearing, vein banding, mosaic, rugosity, stunting,	DQ119041
Ca-CKh19	Khorasan	vein clearing, vein banding, rugosity, stunting, yellowing	DQ870910
Ca-Kh32	Khorasan	vein clearing, vein banding, rugosity, stunting	EF503597
Ca-CSh1	Fars	vein clearing, vein banding, rugosity, stunting, necrosis	DQ119040
Ca-CT4	Tehran	vein clearing, mottling, vein banding, rugosity, stunting, necrosis, leaf deformation	DQ870912
Ca-CT22	Tehran	vein clearing, vein banding, rugosity, stunting, necrosis, leaf deformation	DQ870915
Ca-CQ50	Qazvin	vein clearing, vein banding, rugosity, stunting, necrosis, leaf deformation	DQ870914

Table 2: Symptomatological response of herbaceous indicator plants inoculated with different Iranian *Cauliflower mosaic virus* isolates

		Isolates								
Indicator plant	English name	Ca-CAz22	Ca-CAz26	Ca-CE1	Ca-CKh19	Ca-CKh32	Ca-CQ50	Ca-CSh1	Ca-CT4	Ca-CT22
B. rapa cv. Hakatasuwari	Turnip	NL, VC, M	NL, VC, M	NL, VC, M	NL, VC, M	NL, VC, M	NL, VC, M	NL, VC, M	NL, VC, M	NL, VC, M
B. juncea cv. Hakarashina	Mustard	CLL, VC, M, LD, S	CLL, VC, M, LD, S	CLL, VC, M, LD, S	CLL, VC, M, Ru	ND	CLL, VC, M, Ru	CLL, M, LD	CLL, VC, M	CLL,VC, M, LD
B. napus cv. Otsubu	Oilseed rape	Mo	Mo	Mo	ND	ND	Mo	Mo	Mo	Mo
B. oleracea var. *botrytis*	Cauliflower	VB	VB	VB	ND	VB	VB	VB	ND	ND
B. oleracea var. *gongylodes*	Kohlrabi	-	-	VC, Mo	VC, Mo	-	NL, VC, Mo	CLL	-	-
B. rapa cv. Karaj-01	Turnip	NL, VC, Ru, S	NL, VC, Ru, S	NL, VC, M, S	CL, VC, M, S	NL, VC, M, LD, Ru	NL, VC, M, LD	CL, VC, M, Ru, S	NL, VC, M, LD, Ru	NL, VC, M, LD, Ru
B. rapa ssp. *pekinensis* cv. Kyoto-1go	Chinese cabbage	VC, M, S	VC, M, S	VC, M, LD, S	ND	VC, M, S	VC, M, S	VC, M, S	VC, M, S	VC, M, S
B. rapa ssp. *pekinensis* cv. Kyoto-3go	Chinese cabbage	VC, M, S	VC, M, S	VC, M, LD, S	VC, M, LD, S	VC, M, S	VC, M, S	VC, M	ND	VC, M, S
B. rapa var. *chinensis* cv. Choyo	Qing geng cai	M, S	M, S	M	M	M, LD, S	M, LD, S	VC, M, S	VC, M	VC, M, S
B. rapa var. *chinensis* cv. Tatsuai	Rosette pakchoi	VC, M, Ru	VC, M, Ru	VC, M, Ru	VC, M, Ru	VC, M, S	VC, M, LD, S	VC, M, LD, S	VC, M	VC, M, LD, S
Datura stramonium	Jimpson-weed datura	-	-	-	-	NLL	NL, VC, M	NLL	-	NLL
Eruca sativa	Rocket salad	VC, M	VC, M	ND	M, LD	ND	M	M, S	ND	ND
Hirschfeldia incana	Shortpod mustard	NL, VC, M	NL, VC, M	NL, VC, M, S	ND	ND	NL, VC, M	NL, VC, M, S	NL, VC, M, S	ND
Raphanus sativus cv. Akimasari	Japanese radish	VB	VB	VB	VB	VB	ND	VB	VB	VB
R. sativus cv. Karaj-12	Small radish	VB	VB	VB	VB	VB	VB	VB	VB	VB
Rapistrum rugosum	Rugose rapistrum	VC, M, S	VC, M, S	VC, M, Ru, S	VC, M, Ru, S	VC, M, Ru, S	VC, M, S	VC, M, Ru, S	VC, M, Ru, S	ND
Sisymbrium irio	London rocket	CS, VY, LD, S	CS, VY, LD, S	CS, VY, S	CS, VY, LD, S	ND	CS, VY, S	CS, VY, LD, S	CS, VY, LD, S	VY, S
S. loeselii	Small tumble-mustard	VY, Ru, S	VY, Ru, S	ND	VC, M, Ru, S	ND	VC, M, S	VY, M, Ru, S	VY, M, Ru, S	VC, M, S

CL, Chlorotic Lesions; CS, Chlorotic Spots; LD, Leaf Deformation; M, Mosaic; Mo, Mottling; NL, Necrotic Lesions; NLL, Necrotic Local Lesions; Ru, Rugosity; S, Stunting; VB, Vein Banding; VC, Vein Clearing; VY, Vein Yellowing; ND, Not Determined; -, No Infection

Table 3: List of available *Cauliflower mosaic virus* (CaMV) sequences in GenBank, used for comparative analysis with Iranian isolates

Isolate	Country/location	Source	Accession No.
B29	Rennes, France	*Brassica oleracea* var. *botrytis*	X79465
Bari 1	Bari, Italy	*Diplotaxis tenuifolia*	D00335
BBC	California, USA	*Brassica rapa*	M90542
Cabb B-JI	Wisconsin, USA	*Brassica* sp.	DQ211685
Cabbage S	Bari, Italy	*B. ruvo*	V00141
CM1841	California, USA	*B. campestris*	V00140
CMV-1	California, USA	-	M90543
D-4	California, USA	*B. campestris*	M23620
D/H	Budapest, Hungary	*B. oleracea*	M10376
NY8153	New York, USA	*Brassica* sp.	M90541
PV147	Wisconsin, USA	*B. rapa*	X53860
S-Japan	Yokohama, Japan	*Armoracia rusticana*	X14911
W260	Mendoza prov., Argentina	Unspecified crucifer	L09053
XinJing	XinJiang, China	*B. oleracea*	AF140604

ORF VI sequence, except for small regions on the 3' and 5' ends. DNA was extracted from young turnip infected leaves 30 days after inoculation (Agama *et al*., 2002) and used as template for PCR analyses. Healthy turnip extracts were used as negative controls. PCR amplification was conducted in 50 μL reaction solution containing 5 μL DNA template, 1 μL of each primer (20 pmol μL^{-1}), 5 μL of 10X reaction buffer (200 mM Tris-HCl, 500 mM KCl, pH

8.4), 1.5 μL $MgCl_2$ (50 mM), 1 μL dNTPs (10 mM) and 2.5 units *Taq* DNA polymerase (CinnaGen Inc., Tehran, Iran). Thermocycling was done as follows: 94°C for 2 min, 30 cycles of 94°C for 30 sec, 45°C for 1.5 min, 72°C for 1 min and finally 72°C for 10 min. PCR reactions were done in a Primus (MWG Biotech Co., Germany) thermal cycler. PCR products and DNA ladder (GeneRuler™ 1 kbp DNA Ladder, Farmentas, Lithuania) were fractionated by electrophoresis in 1% agarose gel, in presence of 1 μg mL^{-1} ethidium bromide (Sambrook *et al*., 1989).

Cloning, sequencing and sequence analysis: The amplified DNA for each CaMV isolate under study (Table 1) was cleaned from the amplification reaction mixture using Nucleospin (Macherey-Nagel, Germany) and cloned into pGEM-T Easy vector (Promega Corp., Madison, WI) according to the manufacturers' instructions. Sequences from both strands of the cloned DNA in three independent clones were determined by the custom sequencing service of MWG Biotech Co. (Germany) using standard M13 primer pairs. The sequences obtained were compared among them and with those of other CaMV isolates available in GenBank using Basic Local Alignment Search Tool (BLAST) (Altschul *et al*., 1997) at the National Centre for Biotechnology Information. Multiple sequence alignments were generated by the Clustal W program (Thompson *et al*., 1994). Phylogenetic trees were constructed using MegAlign program ver. 5.00 from the DNASTAR package (DNASTAR, Madison, WI, USA) (Burland, 2000). In this study, the corresponding region of *Horseradish latent virus* (HRLV) was used as outgroup in the alignment of CaMV sequences. For further analysis and to obtain potential restriction enzyme(s) for their differentiation, Iranian and other exotic CaMV isolates (Table 1 and 3) were mapped and compared using Mapdraw program (ver. 5.00, DNASTAR, Burland, 2000).

RESULTS

Serological assays: Almost all cauliflower leaf samples (292/323, corresponding to 90.4% of infection) collected from 32 commercial fields over six provinces surveyed, reacted positively in DAS-ELISA using CaMV-specific antibodies.

Host range and symptoms: The herbaceous host range of the CaMV isolates from cauliflower of different Iranian provinces (Table 1) were substantially similar; some significant differences were observed in the kind of symptoms and in the capacity to infect some discriminating plant species (Table 2). All nine isolates under study systemically infected *Brassica napus* cv. Otsubu (oilseed rape), *B. oleracea* var. *botrytis* (cauliflower), *B. rapa* ssp. *pekinensis* cvs. Kyoto-1go and Kyoto-3go (Chinese cabbage), *B. rapa* var. *chinensis* cvs. Choyo (Qing geng cai) and Tatsuai (Rosette pakchoi), *Eruca sativa* (rocket salad), *Raphanus sativus* cvs. Akimasari (Japanese radish) and Karaj-12 (small radish), *Rapistrum rugosum* (rugose rapistrum) and *Sisymbrium loeselii* (small tumble-mustard) and induced chlorotic/necrotic local lesions and systemic symptoms on *B. rapa* cv. Hakatasuwari (turnip), *B. juncea* cv. Hakarashina (mustard) and *Hirschfeldia incana* (shortpod mustard). On *B. rapa* cv. Karaj-01 (turnip) these isolates induced chlorotic/necrotic lesions 6-15 days after inoculation. Systemic symptoms appeared initially as vein clearing, evolving, according to the isolates, in mosaic, rugosity, leaf deformation and stunting. Only Ca-CE1, Ca-CKh19, Ca-CQ50 and Ca-CSh1 isolates were able to infect kohlrabi plants (*B. oleracea* var. *gongylodes*). Contrarily to Ca-CSh1, which infected only locally this indicator plant, the other three isolates induced systemic vein clearing 2-3 weeks after inoculation. Among the studied isolates, only Ca-CKh32, Ca-CQ50, Ca-CSh1 and Ca-CT22 infected *Datura stramonium* (Jimpson-weed datura). On this host Ca-CKh32, Ca-CSh1 and Ca-CT22 isolates induced only necrotic local lesions, whilst Ca-CQ50 caused also systemic vein clearing and mosaic (Table 2).

Aphid transmission: All CaMV isolates under study were transmitted by *Myzus persicae* on *B. rapa* plantlets, which showed vein clearing, leaf deformation and mosaic symptoms *ca.* 16 days after inoculation. The presence of CaMV in the inoculated plants was confirmed by ELISA.

PCR amplification, sequencing and sequence analysis: In PCR, DNA fragments of approximately 1,580 bp were amplified from all the isolates under study using the primer pair CaMV-6-F and CaMV-6-R. No amplicons were obtained from healthy turnip leaf tissues (Fig. 1). The nucleotide sequences of the amplified fragments for the Iranian CaMV isolates were determined and submitted to the GenBank, with the following accession numbers: DQ119040, DQ119041, DQ870907, DQ870908, DQ870910, DQ870912, DQ870914, DQ870915 and EF503597 (Table 1). BLAST search results confirmed that they contained the complete sequence of the ORF VI, apart from short fragments in the 3' and 5' ends. Multiple nucleotide sequences alignment using the CLUSTAL W program showed 96.9-100% identity among the ORF VI genes of the nine isolates under study. When compared with other non-Iranian CaMV isolates, the Iranian isolates

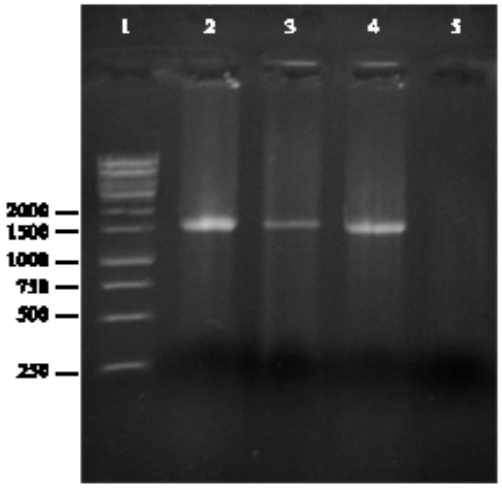


Fig. 1: CaMV detection by PCR using CaMV-ORF VI specific primers. Lane 1, DNA marker (GeneRuler™ 1 kbp DNA Ladder, Fermentas, Lithuania); lane 2-4, positive cauliflower samples (amplification of approximately 1,580 bp); lane 5, negative control

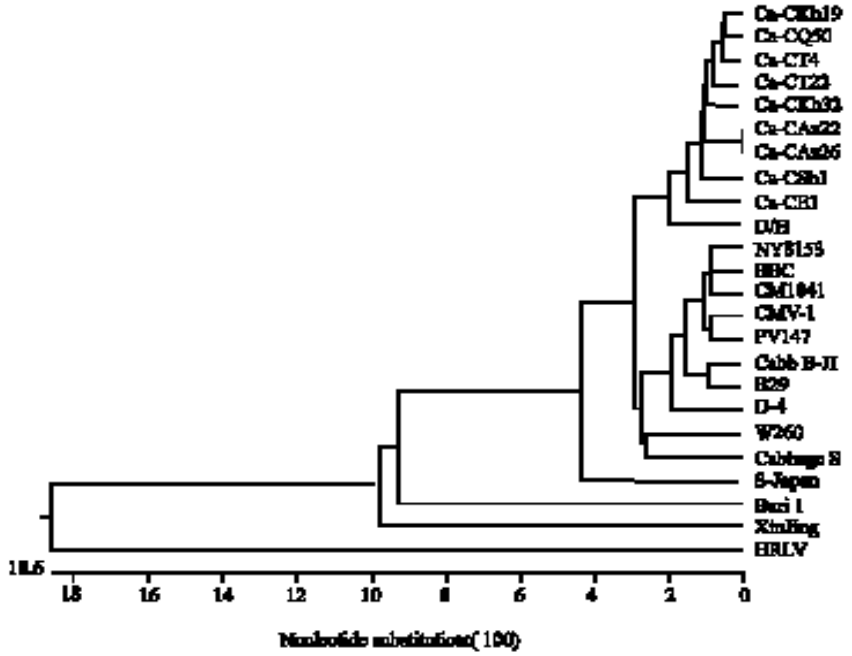
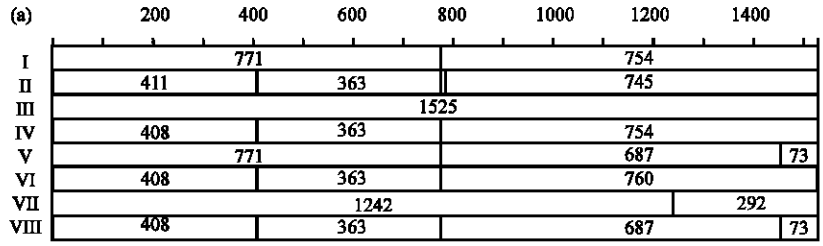


Fig 2: Phylogenetic tree constructed by CLUSTAL W program showing the genetic relationship among ORF VI gene sequences of Iranian and other CaMV isolates

showed the highest and lowest level of identity to D/H (96.1-96.7%) and XinJing (83.7-84.4%) isolates, respectively. In the phylogenetic tree constructed from the nucleotide sequences of the CaMV isolates, all Iranian isolates clustered in the same branch, whereas all North-American isolates as well as B29 clustered in a separate branch. Also, the isolates Cabbage S and W260, S-Japan, Bari 1 and XinJing were located in separate clusters (Fig. 2). For the same ORF, at the amino acid level, there was 96.1-100% identity among Iranian isolates. The deduced amino acid sequences of the Iranian CaMV isolates had the highest and lowest identity to D/H (94.5-95.9%) and XinJing (81.3-82.3%) isolates, respectively.

Restriction Fragment Length Polymorphism (RFLP) profiles of the sequences under study (Table 1 and 3)



(b)

*Hpy*99I RFLP group	Isolates
I	B29, BBC, CM1841, CMV-1, D-4, NY8153, W260
II	Bari 1
III	Cabb B-JI
IV	Cabbage S
V	D/H
VI	S-Japan
VII	XinJing
VIII	Ca-CAz22, Ca-CAz26, Ca-CE1, Ca-CKh19, Ca-CKh32, Ca-CQ50, Ca-CSh1, Ca-CT4, Ca-CT22

Fig. 3: Comparison of Iranian *Cauliflower mosaic virus* (CaMV) isolates with others from different countries on the basis of restriction fragment length polymorphism (RFLP) pattern using *Hpy*99I in Mapdraw program. (a) RFLP profile. The size of restricted fragments (bp) is given between restriction sites. (b) Placement of the isolates in different RFLP groups

were assessed using Mapdraw program. The results showed that with *Hpy*99I eight RFLP groups containing from 0 to 3 restriction sites were created. All nine Iranian isolates were placed separately in RFLP group VIII with three restriction sites at positions 408, 771 and 1458. The majority of North-American isolates together with B29 and W260 isolates were located in RFLP group I, whilst Bari 1, Cabb B-JI, Cabbage S, D/H, S-Japan and XinJing isolates were placed individually in RFLP groups II to VII, respectively (Fig. 3).

DISCUSSION

In this study, CaMV isolates from symptomatic cauliflower plants from different Iranian provinces were selected and analyzed for their biological and molecular properties. Host range studies showed that the isolates under study differed for their reactions especially on turnip, kohlrabi and *Datura* plants, as reported previously for other isolates (Lung and Pirone, 1972; Schoelz *et al*., 1986; Al-Kaff and Covey, 1995). Based on the symptoms expressed on kohlrabi seedlings (*B. oleracea* var. *gongylodes*) the isolates could be separated into three main groups, thus confirming previous reports (Al-Kaff and Covey, 1995). All isolates were able to infect systemically a wide range of cruciferous hosts tested. Some of the isolates infected not only cruciferous plants but included species of the family Solanaceae (*D. stramonium*), on which they induced local or systemic symptoms, as described previously (Lung and Pirone, 1972; Garcia and Shepherd, 1985; Schoelz *et al*., 1986; Schoelz and Shepherd, 1988; Qiu and Schoelz, 1992). All isolates studied in this research were aphid transmissible.

The ORF VI nucleotide sequences of the Iranian CaMV isolates were compared with those of other CaMV isolates available in GenBank. The supposed division of Iranian isolates in two major groups according to their different capacity of infecting cruciferous and solanaceous species did not find a support in molecular comparative analysis of ORF VI genes, confirming previous results (Wintermantel *et al*., 1993; Pique *et al*., 1995). The Iranian isolates shared close identity to a Hungarian isolate from *B. oleracea* (D/H). The phylogenetic analysis among different CaMV isolates based on ORF VI nucleotide sequences did not reveal branching pattern related to the host plant source or aphid transmissibility, as previously reported by Chenault and Melcher (1994b). As indicated by the same authors, the geographic distribution of CaMV sources seems to be the major factor contributing in the evolutionary grouping of this viral species, as the results obtained in this study seem to confirm. The only exception is for B29 isolate from France that clustered with the North-American isolates. The Iranian isolates are more closely related to the Hungarian isolate D/H of CaMV, hence they should be categorized in the non-North American isolates group described previously (Chenault and Melcher, 1994b). RFLP studies performed on the ORF VI gene sequences revealed considerable polymorphism between Iranian and other CaMV isolates. Based on the results obtained, all Iranian isolates were located in a separate RFLP group using *Hpy*99I. Interestingly, the grouping pattern

obtained by using *Hpy*99I strictly agreed with those obtained in phylogenetic studies of ORF VI sequences.

The present study reports cloning, sequencing and comparison of CaMV ORF VI gene of Iranian CaMV isolates for the first time. The phylogenetic results in agreement with restriction analysis revealed that the Iranian isolates can be assigned to a well separated group. This virus was probably successfully introduced in Iran through plant material transportations and diverged in an independent evolutionary way. Further investigations on molecular properties of CaMV isolates in Iran are necessary to confirm this hypothesis.

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