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Phylogenetic Analysis of New Isolates of *Cucumber mosaic virus* from Iran on the Basis of Different Genomic Regions

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Molecular characterization of *Cucumber mosaic virus* (CMV) was done by using samples from tomato and cucurbitaceous plants collected from different locations in the northwest region of Iran. After screening by enzyme-linked immunosorbent assay, 91 CMV-infected samples were identified. Biological properties of eight representative isolates were compared with each other revealing two distinct phenotypes on squash and tomato plants. Phylogenetic analyses based on nucleotide sequences of the coat protein (CP), movement protein (MP) and 2b of the new isolates, together with that of previously reported isolates, led to the placement of the Iranian isolates in subgroups IA and IB according to CP and MP genes, but in subgroup IA according to the 2b gene. These data suggest that reassortment may have been a major event in the evolution of CMV in Iran, and that the Iranian isolates are derived from a common recent ancestor that had passed through a bottleneck event.

Keywords : CMV, CP, genotype, MP, phylogenetic analysis, subgroup, 2b

Cucumber mosaic virus (CMV) is the type member of the genus *Cucumovirus* in the family *Bromoviridae*. CMV has a wide host range in 85 distinct families of dicotyledonous and monocotyledonous angiosperms (Bujarski et al., 2012). The virus is transmitted by some 80 aphid species belonging to 33 genera in a non-persistent non-circulative manner (Palukaitis and Garcia-Arenal, 2003). CMV causes disease outbreaks in tomato, pepper or cucurbits which result in great economic losses for many countries (Gallitelli, 2000). CMV has three single-stranded plus-sense RNAs (RNA1-3) that code for five functional proteins [1a, 2a, 2b, 3a and coat protein (CP)]. Proteins 1a and 2a, encoded by RNA1 and RNA2, respectively, are components of the replicase complex (Hayes and Buck, 1990). The RNA3- encoded proteins, 3a [movement protein (MP)] and CP are involved

in viral movement. The CP is translated from the sub-genomic RNA4 and required for encapsidation, long-distance movement and transmission by aphids (Boccard and Baulcombe, 1993; Perry et al., 1994; Shi et al., 2002). Protein 2b is expressed from RNA 4A, a subgenomic RNA derived from RNA2, is involved in the long-distance movement of CMV in the plant (Soards et al., 2002) and functions as a suppressor of RNA silencing (Ding et al., 1994; Li et al., 1999). Many strains and isolates of CMV have been characterized on the basis of symptoms and host range (Choi et al., 2005; Huppert et al., 2002; Palukaitis and Garcia-Arenal, 2003) and often, differences in such phenotypes were mapped to the genomic RNAs that code for the CP (Shintaku et al., 1992; Sugiyama et al., 2000; Suzuki et al., 1995), MP (Choi et al., 2005) or 2b protein (Du et al., 2007; Shi et al., 2002; Lewsey et al., 2009, 2010).

Phylogenetic studies have resulted in classification of CMV isolates to three subgroups designated subgroup IA (S-IA), subgroup IB (S-IB) and subgroup II (S-II) (Palukaitis et al., 1992; Roossinck et al., 1999). Subgroup I (S-I) and S-II isolates have 75% nucleotide (nt) sequence identity whereas S-IA and S-IB are more closely related and share 92–95% identity (Roossinck, 2001). In a previous study we detected members of S-IA in cucurbits in Iran and analyzed their phylogenetic positions based on the CP sequences (Bashir et al., 2008). Here, we studied some of the biological properties of eight representative CMV isolates on different hosts. In addition to amplification of the MP and CP genes from the collected samples, a specific pair of primers was designed to amplify the 2b gene from Iranian isolates of CMV. The three amplified genomic regions were cloned, sequenced and their phylogenetic relationships were determined. Also, the genotypes of eight representative isolates were investigated in relation to their symptoms on experimental host plants.

Leaves of tomato and cucurbitaceous plants showing symptoms similar to those caused by CMV, were collected from 17 locations in the Ardabil, East Azarbaijan, and West Azarbaijan provinces during 2009–2010. Eight representative CMV isolates from distinct geographical locations

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Fig. 1. Symptoms of representative isolates of *Cucumber mosaic virus* (CMV) from Iran on tomato (A, B, C) and squash (D, E, F) 14 days post inoculation. Severe shoestring by isolate Khn1 (A), mild shoestring by Bon175 (B), mosaic by Bon175 (D), necrosis by Khn1 (E), mock inoculations (C, F).

were selected to investigate their biological properties. Sap from the infected samples was rubbed on Carborundum-dusted leaves of *Chenopodium quinoa*, *Cucurbita pepo* cv. Ps, *Nicotiana benthamiana*, *Nicotiana tabacum* cvs. Coker347, Virginia and Xanthi and *Solanum lycopersicum* cv. Supper using 0.1 M PBS (pH 7.2).

Leaves from collected samples were tested with double-antibody sandwich (DAS)-ELISA (Clark and Adams, 1977). The presence of CMV was detected in 91 out of 339 screened samples with virus like symptoms from different locations in North-West Iran by DAS-ELISA. The infection rates for tomato, cucumber, squash and muskmelon plants were 36%, 30%, 12% and 16%, respectively. The pre-

dominant symptom associated with isolates Bon175, Mgh91 and Jol186 on squash was severe mosaic and in the case of Mgh191, mild mosaic. Isolates Ajs4, Bas3, Esf172 and Khn1 showed necrosis on squash plants 14 dpi, which resulted in death of plants 30 dpi, whereas isolates Bon175, Mgh91 and Mgh191 did not show necrosis even at 35 dpi. In the case of Jol186 only non-lethal necrosis was observed by 35 dpi. Shoestring, a prevalent symptom on tomato, was more severe with isolates Ajs4, Bas3, Esf172 and Khn1, than isolates Bon175 and Mgh91 (Fig. 1A, 1B). Symptoms of Ajs4, Bas3, Esf172 and Khn1, were similar on tobacco cultivars and *N. benthamiana* including mosaic with dark green areas. Isolates including Bon175, Mgh91, Mgh191

Table 1. Characteristics of primers used to amplify different genomic regions of CMV

Primer name	Position	Expected size	Nucleotide sequence ^c
2bIR-F	2226-2252 (2b) ^a	649	5'-TTYGARTTGAAATACARGAAGTCYGGG-3'
2bIR-R	2851-2874 (2b)		5'-CCGTAAGCTGGATGGACAACCCG-3'
CMVCPf	1149-1161 (CP) ^b	867	5'-GCTTCTCCGCGAG-3'
CMVCPr	1998-2015 (CP)		5'-GCCGTAAGCTGGATGGAC-3'
M1-forward	119-138 (MP) ^d	841	5'-CATGGCTTTCCAAGGTACCAG-3'
M2-reverse	938-959 (MP)		5'-CTAAAGACCGTTAACCACCTGC-3'
18s1	357-375 (18S DNA) ^e	461	5'-AACGGCTACCACATCCAAG-3'
18s2	798-817 (18S DNA)		5'-TCATTACTCCGATCCCGAA-3'

^aNucleotide positions correspond to the genomic RNA2 of CMV-Fny

^bNucleotide positions correspond to the genomic RNA3 of CMV-Q (Rizos et al., 1992)

^cR = A or G; Y = C, T

^dNucleotide positions correspond to the genomic RNA3 of CMV-Fny (Lin et al., 2004)

^eNucleotide positions correspond to 18S ribosomal DNA, in the accession JQ612131.1

and Jol186 showed mild mosaic and sometimes no visible symptoms on these plants.

Total RNA from leaves of infected samples was extracted according to Rowhani et al. (1993) with minor modifications as reported previously (Bashir et al., 2008). Three sets of primers were used to amplify different genomic regions of CMV (Table 1). For isolation of the 2b gene a pair of new specific primers was designed by the use of Oligo ver. 5 (Rychlik, 1994). Reverse transcription was carried out with 0.3 pmol 2b, MP or CP specific reverse primers or 5 pmol random hexamer primer (Fermentas, Lithuania) in 20 µl reverse transcription mix. Using specific primers for CP, all the ELISA positive samples (91 samples) were tested with RT-PCR, but amplification with the 2b and MP primers was only performed on 45 samples from different locations which showed different symptoms on the original host plants. The thermocycle program for amplification of the MP or CP cDNAs included 1 cycle of 94°C for 1 min, 30 cycles of 94°C for 30 sec, 50°C for 30 sec and 72°C for 1 min, and a final cycle of polymerization at 72°C for 5 min. Amplification of the 2b cDNA was achieved with a similar thermocycle profile, but the annealing was done at 65°C. RT-PCR with the CP primers on the 91 ELISA-positive samples resulted in amplification of an expected 867 bp fragment from all of them (data not shown). The expected 649 or 841 bp fragment was amplified from the samples tested with the 2b or MP primers, respectively (Fig. 2A, 2B).

PCR products resulting from the use of the CP primers were digested with the cutter *MspI* as described previously (Bashir et al., 2008). Digestion of the amplified CP cDNA with *MspI* produced two fragments with sizes about 537

and 335 bp from each isolate (data not presented). The restriction analysis as a method for typing CMV isolates (Rizos et al., 1992) showed that all the Iranian CMV isolates belonged to S-I. Our previous studies on CMV isolates from Iran have also shown the frequent occurrence of the S-I in the country. While most S-IB isolates are distributed in Asia, S-IA and S-II isolates possess worldwide distribution (Palukaitis and Zaitlin, 1997; Roossinck, 2001).

For subsequent cloning and sequencing procedure seventeen representative PCR products of the 2b gene, 12 of the CP and 10 of the MP gene were selected based on geographical location of the original samples as well as the host plants. Each PCR product (~20 ng each) was ligated into the pTZ57R/T vector (Fermentas, Lithuania). *Escherichia coli* DH5α was made competent according to Chung et al. (1989) and transformed with the ligation mix. The selection and screening were performed as described previously (Bashir et al., 2006). The sequences reported in this paper were submitted to GenBank and assigned the accession numbers JX025971-JX026001, JX000232, and JX00023. Multiple nucleotide sequence alignments of the Iranian isolates with the previously reported CMV strains/isolates (Table 2) were performed by the use of GeneDoc (Nicholas et al., 1997).

The ratio of non-synonymous nucleotide diversity to synonymous nucleotide diversity [Pi(a)/Pi(s)] was estimated by the use of DnaSP version 5.10.01 (Librado and Rozas, 2009). The value of this ratio is 1, < 1 and > 1 under neutral, negative (purifying selection) and positive (directional selection), respectively. The ratio values were determined for different sections of the genomic regions by the use of

Fig. 2. Agarose gel electrophoresis of DNA fragments amplified from some infected and non-inoculated plants by RT-PCR with the 2b (A) and MP (B) primers and 18S rDNA primers as the internal control (461 bp). (A) 100 bp DNA ladder (lane M), non-inoculated control (lane 1), a representative amplification of a 649 bp DNA from isolate Mgh91 (lane 2), amplification with no template (lane 3). (B) 100 bp DNA ladder (lane M), non-inoculated control (lane 1), amplification of an 841 bp fragment from isolates Khn1 (lane 2), Mgh91 (lane 3), Bas3 (lane 4), and without template (lane 5).

Table 2. Accession numbers, abbreviations, host and geographical origins of CMV strains/isolates used for the phylogenetic analyses in this study

Isolate	Accession No.		Host	Origin
	RNA2	RNA3 (MP/CP)		
CK54	–	AF523351 (CP)	<i>Cucurbita pepo</i>	USA
MD284	–	AF523343 (CP)	<i>Solanum lycopersicum</i>	USA
CMV-G10	–	AY541691 (CP)	<i>S. lycopersicum</i>	Egypt
Mi	AB188229	AB188230	<i>Zingiber mioga</i>	Japan
Kor	–	L36251	–	South Korea
Pepo	AB124835	AF103991	<i>C. pepo</i>	Japan
Z	–	AB369269	<i>Nicotiana benthamiana</i>	South Korea
Z1	–	GU327368	<i>C. pepo</i>	South Korea
Licb	AB506799	AB506800	<i>Lilium longiflorum</i>	South Korea
Fuka4-4	AB188232	AB188233	<i>Cucumis sativus</i>	Japan
Cm95	AB188235	Ab188236	<i>N. tabacum</i>	Japan
Y	D12538	D12499	<i>N. tabacum</i>	Japan
D8	AB179765	AB004781	<i>Raphanus sativus</i>	Japan
Mf	AJ276480	AJ276481	<i>Melandryum firmum</i>	South Korea
ZM	JN180310	JN180311	<i>Zea mays</i>	South Korea
RT52	FR827863	AJ810258(CP)	<i>C. pepo</i>	USA
FNY	NC002035	D10538	<i>Cucumis melo</i>	USA
GD	–	HQ916354	<i>Oilseed pumpkin</i>	Austria
Ri-8	AM183118	AM183119	<i>S. lycopersicum</i>	Spain
As	–	AF013291	–	South Korea
K	–	AF127977	–	USA
SD	D86330	AB008777	<i>N. tabacum</i>	China
Ca	AY429433	AY429432	<i>Arachis hypogaea</i>	China
ND2	EU414799	EU414789/EU41486	<i>P. hybrida</i>	China
ND1	EU414798	EU414788/EU41485	<i>N. tabacum</i>	China
TFN	Y16925	Y16926	<i>S. lycopersicum</i>	Italy
Nt9	D28779	D28780	<i>S. lycopersicum</i>	China
PL-1	AM183115	AM183116	<i>S. lycopersicum</i>	Spain
New Delhi	GU111228	GU111229	<i>S. lycopersicum</i>	India
VAL90/1	–	AJ829779(CP)	<i>S. lycopersicum</i>	Spain
Cb-7	DQ785470	EF216867	<i>S. lycopersicum</i>	China
Phy	DQ412731	DQ412732	–	China
Lucknow	–	EF153733	<i>Chrysanthemum morifolium</i>	India
IA	AB042293	AB042294	–	Indonesia
CTL	EF213024	EF213025	<i>Brassica chinensis</i>	China
Ixora	U20218	U20219	<i>S. lycopersicum</i>	Philippines
SNK	FN552598	–	<i>C. sativus</i>	Thailand
–	AJ831395	–	<i>L. longiflorum</i>	India
Bal-In	–	JF279609(CP)	<i>C. melo</i>	India
MaS_Italy	–	JN593376(MP)	<i>Mandevilla sanderi</i>	Italy
Vir56	–	DQ006805(MP)	<i>Thevetia nereifolia</i>	Italy
CMV-UP	–	DQ642017(MP)	<i>Musa sp.</i>	India
S337	–	AY871069(CP)	<i>C. sativus</i>	Iran
SH17	–	AY871068(CP)	<i>C. sativus</i>	Iran
B13	–	AY871070(CP)	<i>C. sativus</i>	Iran
B23	–	AY871071(CP)	<i>C. sativus</i>	Iran
DI1	–	DQ002876(CP)	<i>C. pepo</i>	Iran
EI1	–	DQ002880(CP)	<i>C. pepo</i>	Iran
FI3	–	DQ002883(CP)	<i>C. pepo</i>	Iran
GI1	–	DQ002885(CP)	<i>C. pepo</i>	Iran
Q	X00985	M21464	<i>Capsicum annum</i>	Australia

Fig. 3. Phylogenetic analysis of CMV including new isolates from Iran based on genes encoding the coat protein (A), movement protein (B) or 2b protein (C). Bootstrap values are shown above the branches ($> 70\%$). New Iranian isolates are in bold. Strain Q of CMV, as a subgroup II member, was designated as the outgroup.

sliding window and step-size options of 50 and 10, respectively, in DnaSP. $Pi(a)/Pi(s)$ was < 1 for all three genomic regions; however evidence of positive selection in the CP, MP and 2b genes was found. Nucleotide positions (mid-points) 154–233 in the 2b gene had the ratio value of > 1 with a pick (the highest ratio value of 70.28) (Fig. 4). Although phylogenetic analysis of the Iranian isolates was done previously on the basis of the CP sequences (Bashir et al., 2006 and 2008), here we extended this analysis to the 2b and MP sequences of isolates from different hosts and locations. This is important for studying plant virus evolution and understanding events such as recombination and reassortment that are major evolutionary forces for shaping virus populations including CMV population (Bonnet et al., 2005; Roossinck, 2002).

Phylogenies were inferred by the use of Phylip package version 3.65 (Felsenstein, 2004) and Treecon (Van de Peer and De Wachter, 1997) for parsimony- and distance-based trees, respectively. CMV-Q, a S-II strain, was designated as the outgroup. Finally, the selected tree was viewed by the Treeview program (Page, 1996). Because similar phylogenies were inferred based on the distance or parsimony method, regardless of whether nucleotide or amino acid data were used, only distance trees based on the nucleotide data are presented here. In the phylogenetic tree based on nucleotide sequences of the CP gene (Fig. 3A) the newly sequenced isolates of the present research including Bon175, Bon94, Jol186, Mgh91, Mgh191, Sh40, Sh44, and Zdj31, were placed in the S-IA cluster sharing 93–99% nt (and 94–100% aa) identities with other S-IA isolates. These isolates, except for Jol186, together with two American isolates, CK54 and MD284, formed a subclade radiating from the S-IA clade, with the highest nucleotide identity of 99% to the American isolate CK54. Previously reported CMV isolates from Iran such as DI1, EI1, FI3, GI1 (Bashir et al., 2008) were also placed with these isolates sharing 95–99% nt (and 98–99% aa) identities with them. Jol186 was placed with the remaining previously reported Iranian isolates B13, B23, S337 and SH17, (Bashir et al., 2006) in a different subclade with 99% similarity to ZM (Korean isolate). Isolates Ajs4, Bas3, Esf172 and Khn1 together with Bal-In isolate from India formed a subclade radiating from the S-IB clade sharing 99% identity to isolate CMV-G10 from Egypt. Isolates (Ajs4, Bas3, Esf172, and Khn1) that were placed in S-IB clade on the basis of the biological studies were apparently more virulent than (Bon175, Mgh91, Mgh191 and Jol186) isolates in S-IA clade. Isolates in S-IA clade induced mosaic on squash, mild shoestring on tomato and mild mosaic on tobacco cultivars and *N. benthamiana*. This is also in agreement with a previous report that S-IB isolates are more virulent than S-IA isolates (Du et al., 2007). The CP nucleotide and amino acid identities of

isolates characterized in this study and that of other S-IB isolates were both 91–99%.

Distribution of CMV isolates into different subgroups on the MP-based phylogenetic tree (Fig. 3B) agreed with that in the CP-based tree. Iranian S-IA isolates had the highest similarity (99%) with isolate Pepo. Isolate Jol186 with 99% identity to several non-Asian isolates, such as the Italian isolate Ri-8, formed a subclade within the S-IA clade, in the most distal position to the Iranian isolates. The nucleotide and amino acid identities of the MP region of the newly characterized isolates to other S-IA isolates were 96–99% and 99–100%, respectively. Similar to what was seen in the CP-based phylogenetic tree, isolates Ajs4, Bas3, Esf172 and Khn1 with 92–96% nt (94–97% aa) identities to other isolates were placed in S-IB. The closest previously reported isolate to these Iranian isolates was CMV-UP with 96% identity. In the phylogenetic tree based on the 2b gene (Fig. 3c), 29 out of 45 analyzed isolates including seventeen Iranian isolates were placed in S-IA with 90–99% nt identities to previously reported S-IA members. However, in the MP- or CP-based tree these isolates were divided into S-IA and S-IB. The most similar strain to the Iranian isolates in the 2b gene was Fny with 99% nt identity. The nucleotide and amino acid identities between the newly reported Iranian isolates were 91–99% and 94–100% for the CP gene, 92–99% and 96–100% for the MP gene, and 98–99% and 98–99% at the 2b gene.

When nucleotide sequences were used as the basis of the analysis, subgroup affiliation of all the previously reported isolates remained the same on the CP-, MP- or 2b-based trees with the exception of isolate D8. This isolate belonged to S-IA on the CP- and MP-based trees but to S-IB on the 2b-based tree possibly because of reassortment in CMV isolates, a phenomenon which has already been reported in strains of CMV (Lin et al., 2004; Liu et al., 2009; Roossinck, 2002). All the Iranian isolates characterized in this study were divided into S-IA (6 isolates) and S-IB (4 isolates) on the basis of CP and MP sequences, but were placed in S-IA on the 2b tree. This suggested that the 2b gene does not co-evolve with the other genomic regions.

Generally, the evolution rate of the 2b gene is known to differ from the other genomic regions (Liu et al., 2009; Lin et al., 2004). However, others (Liu et al., 2009; Roossinck, 2002) argued that the 2b was the most diverse genomic part of CMV. $Pi(a)/Pi(s)$ for the 2b genes of Iranian isolates was even less than that of the MP and CP genes. However, a section of the 2b region for the Iranian isolates, amino acid positions 52–78 proximal to the section reported for group α , was found to possess the ratio value of greater than 1 (Figure 4). Therefore, the Iranian CMV population appears similar to group α isolates of California (Lin et al., 2004) in respect to the diversity of the 2b gene. We speculate that

Fig. 4. A curve representing evidence of positive selection along alignment of the 2b gene from seventeen CMV isolates from Iran. The $Pi(a)/Pi(s)$ ratios were generated by DnaSP version 5.10.01 by the use of sliding window and step size options of 50 and 10, respectively. The region between nucleotide positions (midpoints) 154 and 233 has the ratio value of > 1 . The highest value (70.28) belongs to position 210.

such isolates might have passed through a bottleneck when the virus was introduced into the country and thus resulted from a founder effect by a common ancestor that colonized and spread only recently in the region.

It also appeared that the Iranian isolates, in the particular the S-IB isolates, had the highest similarities to the Asian isolates of CMV on the basis of the CP and MP sequences. Most S-IB isolates are distributed in Asia whereas S-IA and S-II are found worldwide (Palukaitis and Zaitlin, 1997; Roossinck, 2002). On the other hand, the Iranian S-IA isolates had maximum similarities to the USA isolates, therefore, it may be concluded that the Iranian isolates have different origins. Some of them including Ajs4, Bas3, Esf172 and Khn1 which belong to S-IB, probably originated in Asia and evolved further in Iran. These isolates appear to have evolved earlier than other Iranian isolates as shown by their closer phylogenetic positions to S-II (Fig. 3). Thus, S-IB isolates are presumed to be ancestors for S-IA isolates (Roossinck et al., 1999). Iranian S-IA isolates have probably originated from different locations other than Asia and then spread through seeds in Iran.

In summary, the outcome of this study drew a tentative link between genotypes and biological properties of the representative isolates. The frequent occurrence of S-I members was again demonstrated. Phylogenetic positions of the newly characterized isolates remained the same

Table 3. Characteristics of new Iranian isolates of CMV reported in this study

Isolate ^a	Origin	Phenotype ^b	PCR Detection			Subgroup ^c	Phylogenetic position		
			2b	cp	mp		2b	cp	mp
Orm	Orumieh	n.t.	+	+	n.t. ^d	I	IA ^e	n.t.	n.t.
Jol186	Jolfa	SM	+	+	+	I	IA	IA	IA
Bon94	Bonab	n.t.	+	+	+	I	IA	IA	IA
Mgh91	Maragheh	SM	+	+	+	I	IA	IA	IA
Mgh205	Maragheh	n.t.	+	+	n.t.	I	IA	n.t.	n.t.
Bon175	Bonab	SM	+	+	+	I	IA	IA	IA
Esf172	Esfahalan	N	+	+	+	I	IA	IB	IB
Mgh191	Maragheh	MM	+	+	+	I	IA	IA	IA
Sh201	Shabestar	n.t.	+	+	+	I	IA	n.t.	n.t.
Zdj31	Zanjirabad	n.t.	+	+	+	I	IA	IA	IA
Mia142	Mianeh	n.t.	+	+	+	I	IA	n.t.	n.t.
Bas3	Basmenj	N	+	+	+	I	IA	IB	IB
Bon2	Bonab	n.t.	+	+	+	I	IA	n.t.	n.t.
Khn1	Khodafrin	N	+	+	+	I	IA	IB	IB
Mgh1	Maraghe	n.t.	+	+	+	I	IA	n.t.	n.t.
Ajs4	Ajabshyr	N	+	+	+	I	IA	IB	IB
Adb68	Ardabil	n.t.	+	+	n.t.	I	IA	n.t.	n.t.
Sh40	Shabestar	n.t.	n.t.	+	+	I	n.t.	IA	n.t.
Sh44	Shabestar	n.t.	n.t.	+	+	I	n.t.	IA	n.t.

^a isolates Orm, Bon94, Esf172, Bon2, Khn1, Mgh1, Ajs4 and Adb68 were from tomato; Jol186, and Mgh91 from muskmelon; Mgh205, Bon175, Sh201, Zdj31, Bon2 and Bas3 from cucumber; Mgh191, Sh40 and Sh44 from squash

^b Symptoms on squash and tomato test plants, SM = severe mosaic on squash and no or mild shoestring on tomato, N = necrosis on squash with severe shoestring on tomato, MM = mild mosaic on squash and tomato

^c Determined by *MspI* digestion of the PCR products resulting from the use the CP primers.

^d n.t. = not tested.

^e IA and IB refer to CMV subgroups IA and IB

regardless of whether MP or CP sequences were used, but differed when the 2b sequences were set as the basis of the analysis. Results from this study suggested that the Iranian isolates are derived from a common recent ancestor that had passed through a bottleneck event.

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