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## First Report of Coat Protein Sequence of Cucumber Green Mottle Mosaic Virus in Cucumber Isolated from Khorasan in Iran

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### ABSTRACT

A virus was isolated from cucumber showing mottle mosaic during growing seasons of 2009 and 2010 in Khorasan province of Iran. Based on its serological relationships, biological reactions, Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using specific primers and nucleotide sequence analysis of Coat Protein (CP) gene, the isolated virus was identified as Cucumber Green Mottle Mosaic Virus (CGMMV) and designated as kalat isolate (CGMMV-kalat). Crude sap from infected tissue was mechanically transmitted to various indicator plants which produced characteristic symptoms of tobamovirus infection. However, no symptom was observed in *Petunia hybrid*, *Datura stramonium* L., *Nicotiana* sp. and *Cucurbita pepo*. In RT-PCR assay with specific primers for detection of CGMMV, a single band of about 490 bp in length was produced from the samples. The amplified DNA was cloned and the nucleotide sequence was determined. Sequence comparisons with the CP gene of other CGMMV isolates present in the GenBank indicated that CGMMV-kalat had high homology with the other isolates (the highest homology could reach 97.9% in nucleic acid level). As well, phylogenetic tree based on CP amino acid sequences was established and the highest amino acid homology was 95.2%. To our knowledge, this is the first report on the occurrence of CGMMV in cucumber in Iran based on biological reactions, RT-PCR and CP gene analyses.

**Key words:** Cucumber green mottle mosaic virus, RT-PCR, cloning, coat protein sequence, phylogeny

### INTRODUCTION

Virus infections are a major limiting factor in cucurbit production in Iran. Cucumber green mottle mosaic virus belongs to the genus *Tobamovirus* and the family *Virgaviridae* that infects many cucurbit species in the world and causing mottle and systemic mosaic symptoms on cucurbitaceous plant leaves (Kim *et al.*, 2003). Tobamoviruses are readily spread mechanically within the field by handling and mechanical damage to plants but not by insect or by fungal vectors (Choi *et al.*, 2002). The nucleocapsid of CGMMV has a rod shape with dimensions nearly of 300-320×18 nm; its genome is a positive-sense, single-stranded RNA of around 6450-nt long which contains four Open-Reading Frames (ORFs) encoding four proteins: two replicase proteins (130 and 180 kDa), one movement protein (MP, 30 kDa) and one coat protein (CP, 17 kDa) (Tan *et al.*, 2000; Lewandowski, 2000) rod-shaped particles of virus are very stable and simple. Virus capsid is not enveloped. CGMMV was first described in cucumber and named cucumber virus 3 (CV3) and cucumber virus 4 (CV4) by Ainsworth (1935) and Ugaki *et al.* (1991). Several strains of CGMMV have been reported from Europe, Israel, Korea, Pakistan, India and Japan (Lee, 1996).

Most strains have a narrow host range limited to family Cucurbitaceae. (Antignus *et al.*, 2001). The Cucurbitaceae family includes several species of horticultural crops that have great economical important like watermelon, cucumber, squash and melon (Gill *et al.*, 2010). Iran cultivates the second-largest total cucurbit acreage (770,000 ha) in the world, exceeded only by China and is the third-largest producer of cucurbits after China and Turkey. Cucurbits in greenhouses or under plastic covers are grown in most areas of Iran, with cucumber accounting for approximately 98% and watermelon and melon for 1.5% and 0.5% of the total acreage, respectively (Massumi *et al.*, 2007). The cucumber (*Cucumis sativus*) is a widely cultivated plant in the gourd family Cucurbitaceae (Ayyappan *et al.*, 2006; Qureshi *et al.*, 2000) and grown in most temperate countries. It is estimate that more than a million hectares of cucumbers were planet worldwide, yielding approximately 23 million metric tons of fresh products (Onto *et al.*, 2008). Among the Cucurbitaceous crops cucumber is sensitive to many viruses and shows a more severe reaction to viruses (Lovisol, 1980).

Although CGMMV is widespread in the world, there are few reports on its occurrence in Iran. CGMMV was reported in Markazi province by Ghorbani (1986) and Safaeizadeh (2008) it has not been reported in Khorasan province (North-East of Iran) until now; also a report from genetic sequence of CGMMV isolates has not yet been published in Iran. ELISA and RT-PCR are methods commonly used to detect CGMMV (Liu *et al.*, 2009). In addition, several molecular techniques have been widely used for the diagnosis of plant viruses, allowing the detection of very small amounts of a virus and also the cloning of genomic fragments of viruses (Henson and French, 1993). The aims of this study were to study the existence and spread of CGMMV in cucurbitaceous growing areas in North-East of Iran and to identify CGMMV at the molecular level.

## **MATERIALS AND METHODS**

**Virus sources:** Samples were collected from June 2009 to October 2010 from different cucurbit plants including cucumber, bottle gourd, zucchini squash, watermelon (*Citrullus lanatus*) and melon (*Cucumis melo* varieties including Cantaloupe, snakemelon, longmelon and local cvs.) from fields and cucumber greenhouses in major cucurbit growing areas of Khorasan province (Torbat-e-Jam, Neyshabour, Mashhad, Chenaran, Kashmar, Kalat, Sabzevar) in Iran. Samples (n = 198) consisted of young fully expanded leaves showing Symptoms such as mottling and systemic mosaic, mild to severe yellow mottling, vein clearing and blistering.

**Double-Antibody Sandwich (DAS)-ELISA:** DAS-ELISA was performed on Cucurbit leaf samples following the general protocol of Clark and Adams (1977). The commercial antiserum (SEDIAG S.A, Strasbourg, France) and conjugate were used at 1:200 dilutions. Wells of micro titer plates were coated with 100  $\mu$ L of each immunoglobulin G in carbonate coating buffer (0.06 M Na<sub>2</sub>CO<sub>3</sub> and 0.14 M NaHCO<sub>3</sub>, pH 9.6) and incubated at 37°C for 4 h; the assays were carried out according to the manufacturer's materials and methods. Extracts from healthy *C. sativus* were used as negative control in all tests. Reactions were measured spectrophotometrically at 405 nm using an ELISA plate reader (STAT FAX 2100, USA). A sample was considered virus-positive if the Optical Density (OD) exceeded the mean plus three standard deviations of the OD of the healthy controls. A typical CGMMV isolate (cv4) was used as reference virus in the serological, biological and molecular detection testes. Samples that showed high ELISA values were selected for RT-PCR analysis.

**Host reaction:** Virus isolation biologically was carried out using single local lesion assay following three passages onto *Chenopodium amaranticolor* Coste and Reyn. Then virus was propagated in

*C. melo*. For definition of the host range, ELISA positive samples were homogenized in 50 mM potassium phosphate buffer (pH 7.0) and the extract was rubbed onto leaves dusted with carborundum powder. At least two plants of each of the following species were inoculated: *C. amaranticolor*, *Petunia hybrida*, *Datura stramonium* L., *Cucumis sativus*, *C. melo* var. *flexuosus*, *Citrullus lanatus* Thunb.cv. Crimson sweet. *Cucumis melo* L. (Longman, local cvs., cantaloupe cv. Honeydew), *C. melo* var. *flexuosus* (snakemelon), *Cucurbita pepo* (squash cvs. Black knight and Local) and *Nicotiana* sp. plants were maintained in an insect-proof screen house at 22-27°C and observed at least 14 days after inoculation. ELISA test was used addition to symptoms observation for checking the presence of the virus particularly in the case of symptomless infections.

**Total RNA isolation and RT-PCR:** Leaf tissue (100 mg) was ground into fine powder in liquid nitrogen and then transferred to microtube. Total RNA was extracted from pulverized tissue using AccuZol™ Reagent (Bioneer, Alameda, CA) according to the manufacturer's instructions. After precipitating with ethanol, total RNA was re-solubilized in 25 µL of RNase-free water.

The complete sequence of the SH strain of CGMMV (CGMMV-SH. GenBank accession number D12505) was used for designing Forward and Reverse primers. Forward primer (5'-ATGGCTTACAATCCGATCAC-3', at Positions 5763-5782) and reverse primer (5'-CTACCACCTCGAAAGCTTAG-3', at Positions 6229-6248) covered a full CP gene. Reverse Transcription (RT) reaction was performed as follows: 1 µL of reverse primer (20 picomoles) and 1 µL of RNA sample were added to 8.5 µL of diethyl pyrocarbonate (DEPC)-treated water. The mixture was incubated at 65°C for 10 min and chilled on ice for 3 min to denature the RNA. Then 3.5 µL of DEPC-treated water, 4 µL of 5×M-MLV RT buffer, 2 µL of dNTPs mix (10 mM) and 1 µL M-MLV(200 U µL) reverse transcriptase (Promega, USA) were added to mixture. The RT reactions were incubated at 42°C for 60 min followed by 95°C for 3 min to terminate the RT reaction. Viral cDNA was then amplified by PCR. The PCR reaction was performed using 2.5 µL of cDNA, 13.5 µL DEPC-treated water, 5 µL of 5×GoTag polymerase buffer, 2.5 µL 10×MgCl<sub>2</sub>, 0.5 µL of each forward and reverse primers (20 pmol), 0.75 µL of dNTP mix (10 mM) and 0.125 µL of GoTag polymerase (2.5 U µL) (Promega, USA). Following program was used for PCR: A first denaturation for 3 min at 94°C was performed and followed by 35 cycle of denaturation for 45 s at 94°C, 30 sec of annealing at 60°C, extension for 1 min at 72°C and a final extension step at 72°C for 7 min. PCR products were analyzed by electrophoresis in 1.2% agarose gel and visualized by ethidium bromide staining.

**Cloning and sequencing:** RT-PCR products of CGMMV isolates were then purified from 1% agarose gels using the DNA gel extraction kit (bioneer, korea) and cloned into the pDrive vector using Inst/Aclon PCR product cloning kit (Qiagen, USA) according to the manufacturer's instructions and transformed into *Escherichia coli* strain DH5α. Plasmid DNA from recombinant clones was purified using the high pure plasmid isolation kit (Roche, Germany) and nucleotide sequencing reactions were performed by MWG Company (Biotech, Germany) using dideoxy nucleotide chain termination method with M13-forward and reverse primers. This isolate and 19 isolates from other parts of the world present in GenBank were analyzed to determine their phylogenetic relationship.

**Phylogenetic analysis:** The phylogenetic analysis of the Iranian isolate was conducted by comparing the 486 bp of the CP gene with those of the other isolates of CGMMV obtained from GenBank (Table 1). Nucleotide sequence analysis and translation to the corresponding amino acid

Table 1: GenBank accession numbers and origin of previously reported CGMMV Isolates/strains<sup>a</sup> used for phylogenetic comparison of the 486 nucleotides long fragment of genome including CP gene

Isolate or strain	Accession no.	Host	Origin	No. of nucleotides
CGMMV-W	AB015146	Watermelon	Japan	6424
CGMMV-SH	D12505	Watermelon	Japan	6424
CGMMV-Y	DQ399708	Watermelon	South Korea	524
CGMMV-KOM	AF417243	Oriental melon	South Korea	6424
CGMMV-KW	AF417242	Watermelon	South Korea	6424
CGMMV-W(kor)	AF225984	Watermelon	South Korea	634
CGMMV-GR3	AJ459421	Watermelon	Greece	486
CGMMV-GR7	AJ459423	Watermelon	Greece	486
CGMMV-GX-G	DQ647384	Cucumber	China	654
CGMMV-LHP	DQ997778	Cucurbits seed	China	524
CGMMV- Bottle gourd	DQ767636	Bottle gourd	India: Rajasthan	486
CGMMV-AL1	AJ748352	Bottle gourd	India	486
CGMMV -MC-1	EF521882	Cucumber	Russia	484
CGMMV- MC-2	GQ495274	Cucumber	Russia	6422
CGMMV-pak	AB127937	Bottle gourd	Pakistan	1012
CGMMV-zucchini	AB194531	Zucchini	Indonesia	486
CGMMV -SP	GQ411361	Cucumber	Spain	6422
CGMMV- Ukraine	FJ426406	Cucumber	Ukraine	542
CGMMV-BG	FJ654659	Bottle gourd	Taiwan	6422
CGMMV-kalat	HQ329106	Cucumber	Iran	486
KGMMV(out group) <sup>b</sup>	AF239212	Zucchini	Korea	1102

<sup>a</sup>: The CGMMV isolates were identified by location, plant host and series number. <sup>b</sup>: KGMMV is Kyuri green mottle mosaic virus included as outgroup

sequence were performed using DNAMAN software (version 4.02) package (BBA, Germany). The nucleotide and deduced amino acid sequences were compared with the equivalent sequences of CGMMV. Multiple alignments of the nucleotide and amino acid sequences were carried out using the ClustalW program in BioEdit (version 7.0.9.) software and DNAMAN software (version 4.02). Phylogenetic trees for grouping based on nucleotide and amino acid sequences were constructed by MEGA 4.1 software program using similarity matrix and the neighbor-joining method. Tree branches were bootstrapped with 1000 replications and bootstrap values less than 50 % were condensed on nodes (Fig. 4).

## RESULTS

**Virus isolate:** Among 198 samples collected from different areas of Khorasan province, 39 samples were positive in ELISA test (The rate of infection was 19.7%). Positive samples including 1 sample from bottle gourd, 5 samples from melon, 0 from zucchini squash, 8 from cucumber in fields and 18 from cucumber in greenhouses, 3 from watermelon and 4 from cantaloupe, were selected for further studies. The virus often found in cucumber greenhouses to fields. The symptoms consisted of sever mottling and varying degrees of mosaic (Fig. 1) and in many cases mix with other cucurbit infecting viruses (data not shown).

**Host range:** Under greenhouse conditions kalat-CGMMV isolate caused systemic symptoms example sever mottle mosaic in cucumber and melon cultivars, leaf mottling and blistering in watermelon. Yellowing and systemic vein clearing in the first true leaf about three weeks post

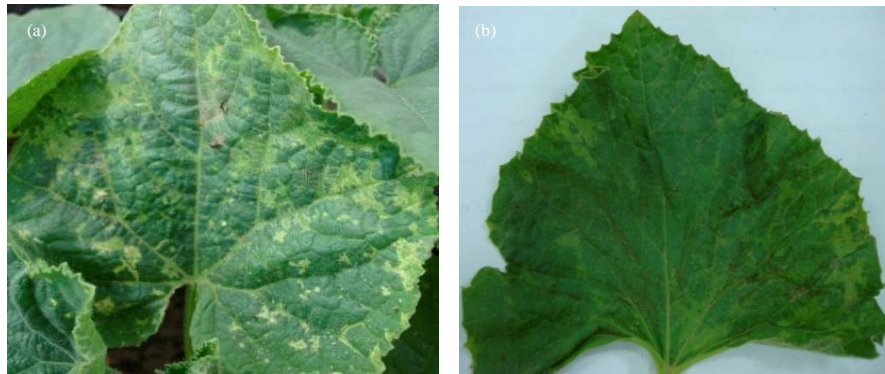


Fig. 1(a-b): CGMMV symptom on cucurbit plants. (a) Severe mottle mosaic on greenhouse cucumber leaf blade and (b) mottle mosaic of melon leaf in field



Fig. 2 (a-d): Symptoms of CGMMV on a number of indicator plants in greenhouse condition (a) chlorotic local lesion on *C. amaranticolor*, (b) mosaic and blistering of *Citrullus lanatus* leaf, (c) and (d) severe mottle mosaic on *C. sativus* and *C. melo*, respectively

inoculation were observed in *C. melo* var. *flexuosus*. Chlorotic spots lesions in the inoculated leaves were produced on *C. amaranticolor* (Fig. 2). No symptoms were observed on *Petunia hybrid*, *Datura stramonium* L., *Nicotiana* sp. and *Cucurbita pepo*. Similar results were observed when inoculated leaves of these plant species were checked by DAS-ELISA but some of ELISA positive

samples were without symptoms. In Khorasan province, we detected CGMMV in only cucurbit plants and CGMMV has not been detected in non-cucurbit plants. Attempts to isolation of virus from melon and watermelon in the field were unsuccessful.

**RT-PCR, sequence data and identity matrix:** In order to provide a fast and sensitive detection method in addition to host reaction and serological tests for the diagnosis of disease caused by CGMMV, a RT-PCR method was developed. The PCR-amplified of approximately 490 bp was obtained (Fig. 3a) by using specific primers CGMMV-F and CGMMV-R, when RNA extracted from isolates was used as the template for the first- strand cDNA synthesis. However, some samples were evaluated as positive in ELISA test but no DNA fragments were amplified from total RNAs extracted from this samples. Anyway, the RT-PCR products were cloned into pDrive vector (3.8 kb) (Fig. 3b) and one of them sequenced. Sequence information for kalat isolate has been submitted to NCBI-GenBank with the accession number HQ329106. The identity of CP nucleotide sequence of kalat isolate in comparison with other GenBank isolates of CGMMV ranged from 89 to 98%. The Iranian isolate in group IA displayed the lowest (88.9%) and the highest (97.9%) nucleotide identity sequence with Ukraine and W-Japan, respectively (Table 2).

**Phylogenetic analysis:** Phylogenetic tree based on multiple sequence alignment of CP divided all CGMMV isolates into two large groups: group I included 2 subgroups A, B (Fig. 4a). Isolates in group II included European isolates (Spain, Ukraine, Greece and Russia). Group I included Asian isolates and GR7 isolate from Greece. Iranian isolate (kalat) with Japan, China, Korea, India,

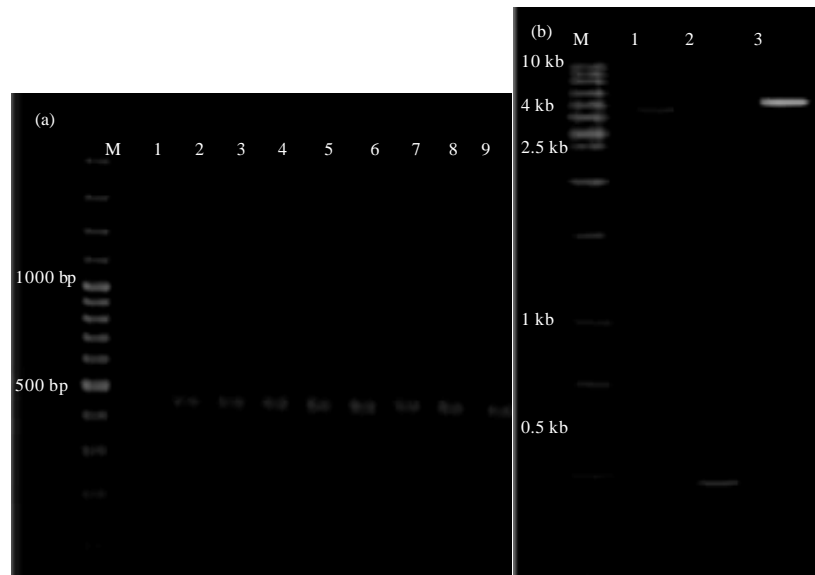


Fig. 3 (a-b): (a) Electrophoresis pattern of DNA fragments amplified by RT-PCR in 1.2% agarose gel related to seven selected CGMMV isolates collected from different location of Kahorasan province (lanes 3-9) 1: healthy cucumber plant extract as negative control. 2: CV4 isolate. M: 100 tp DNA marker (Ferments) and (b) M: 1-kb DNA ladder, lanes 1-3, represent pDrive vector (3.85 kb), CP amplified gene of kalat isolate (486 bp), transformed fragment (about 4.25 kb), respectively

Table 2: Comparison of the percent nucleotide (top and right) and amino acid (bottom and left) identities of 486 nt CP sequences between selected CGMMV isolates<sup>a</sup> used in this study

	Kalat	Pak	AL1	Y	W (kor)	GX-G	LHP	SH	W (Jap)	BG	Zucchini	GR3	GR7	SP	MC-1	MC-2	Ukraine	Bottle-g	KW	KOM
1	*	97.1	97.7	96.9	97.3	97.3	97.3	97.3	97.9	97.1	97.5	90.9	97.5	90.3	91.3	91.3	88.9	97.7	97.1	97.3
2	93.9	*	98.1	97.3	98.1	97.7	97.7	98.3	98.3	97.5	97.9	91.5	97.7	90.9	91.9	91.9	89.3	97.5	97.5	97.7
3	95.2	97.2	*	98.7	99.1	98.3	99.1	98.9	98.9	98.3	99.3	91.9	99.1	92.3	98.3	92.3	89.7	98.9	98.9	99.1
4	93.2	95.2	97.9	*	99.1	97.9	99.5	99.1	98.5	98.9	99.3	91.7	98.3	90.7	91.7	91.7	89.1	98.1	99.3	99.1
5	94.5	96.5	99.3	97.9	*	97.9	99.5	99.5	99.5	98.5	99.3	98.5	92.5	98.7	91.5	92.5	89.9	98.5	99.3	99.5
6	93.2	90.5	96.5	95.2	95.9	*	98.3	97.9	99.3	97.7	98.1	91.3	97.9	90.7	91.7	91.7	89.1	97.7	98.1	97.9
7	93.9	91.2	98.6	97.2	99.3	99.3	*	99.5	98.9	99.3	99.7	92.1	98.7	91.1	92.1	92.1	89.5	98.5	99.7	99.5
8	93.9	95.9	98.6	97.2	97.2	99.3	95.2	*	98.5	99.3	99.7	92.1	98.7	91.1	92.1	92.1	89.5	98.5	99.3	99.5
9	94.5	97.9	97.9	96.5	97.2	97.2	97.9	*	98.3	98.3	98.7	91.5	98.5	90.9	91.9	91.9	89.3	98.3	98.7	98.3
10	93.9	95.9	98.6	97.2	97.9	99.3	95.2	98.6	98.6	*	99.5	91.9	98.5	90.9	91.9	91.9	89.3	98.3	91.9	98.3
11	94.5	96.5	99.3	97.9	98.6	100	95.9	99.3	99.3	97.2	*	92.3	98.9	91.3	92.3	92.3	89.7	98.7	99.5	99.7
12	87.9	88.5	89.8	89.1	89.1	90.5	89.1	89.8	89.9	89.1	89.8	*	92.1	95.8	97.5	97.5	94.4	91.1	92.1	92.3
13	94.5	95.9	98.6	96.5	97.9	95.2	97.2	97.2	96.5	97.2	97.9	89.1	*	91.5	92.5	92.5	89.9	98.5	98.5	98.7
14	82.5	83.1	84.4	83.7	83.7	85.1	83.7	84.4	84.5	83.7	84.4	85.1	90.4	*	98.3	98.3	95.6	90.5	91.1	91.3
15	86.5	87.1	88.5	87.8	89.1	87.8	88.5	88.5	87.8	88.5	89.1	95.2	87.8	95.2	*	100	96.7	91.5	92.1	92.3
16	86.5	87.1	88.5	87.8	89.1	87.8	88.5	88.5	87.8	88.5	89.1	95.2	87.8	95.2	100	*	96.7	91.5	92.1	92.3
17	80	80.5	81.8	81.2	81.2	82.5	81.2	81.8	82	81.2	82.5	87.8	81.2	88.5	91.2	91.2	*	88.9	89.5	89.7
18	94.5	94.5	97.2	95.9	96.6	93.9	95.9	95.9	95.2	95.9	96.6	87.9	95.9	82.5	86.5	86.5	80.0	*	98.3	98.5
19	93.2	95.9	97.9	98.6	98.6	95.9	99.3	97.9	97.2	97.9	98.6	89.8	96.5	84.4	88.5	88.5	81.8	95.2	*	99.3
20	94.5	96.5	99.3	98.6	100	95.9	99.3	99.3	97.2	99.3	100.0	90.5	97.9	85.1	89.1	89.1	82.5	96.6	98.6	*

<sup>a</sup>: Isolates and strains are designated as 1: Kalat (Cucumber: Iran), 2: Pak (Pakistan), 3: AL1 (Bottle gourd: India), 4: Y (South Korea) 5: W (watermelon: Korea), 6: GX-G (Cucumber: China), 7: LHP (Cucurbits: China), 8: SH (watermelon: Japan), 9: W (watermelon: Japan), 10: BG (Bottle gourd: Taiwan), 11: Zucchini (Indonesia), 12: GR3 (watermelon: Greece), 13: GR7 (watermelon: Greece), 14: SP (Cucumber: Spain), 15: MC-1 (Cucumber: Russia), 16: MC-2 (Cucumber: Russia), 17: Ukraine (Cucumber: Ukraine), 18: Bottle-g (Bottle gourd: India), 19: KW (Watermelon: Korea), 20: KOM (Oriental melon: Korea).



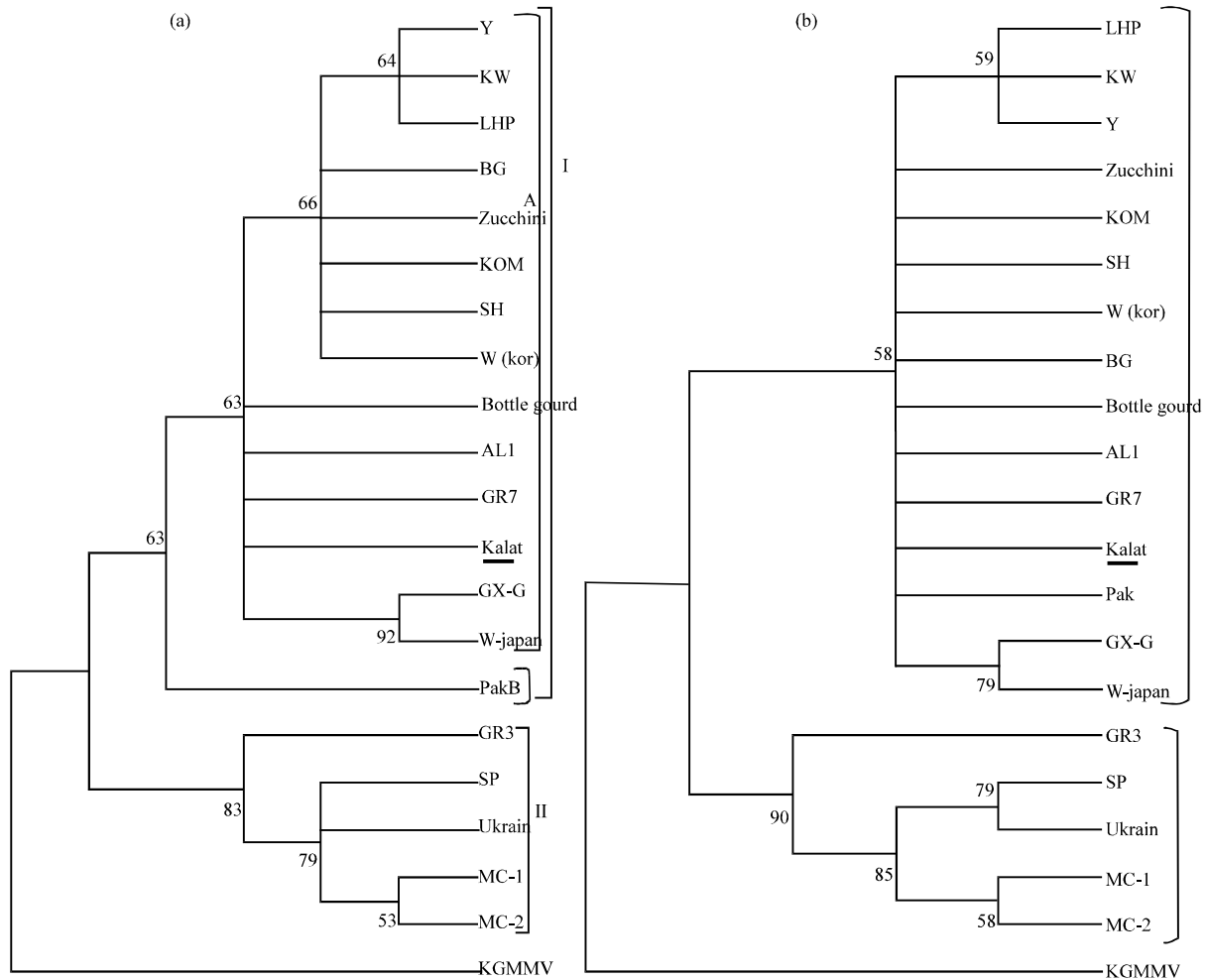


Fig. 4 (a-b): Phylogenetic tree constructed from the alignment of (a) nucleotide and (b) amino acid sequences of coat protein gene of 20 CGMMV isolates (Table 1) using neighbor-joining method based on 1000 replicates. The numbers indicate bootstrap percentage. Bootstrap values higher than 50 are indicated on nodes and the nodes less than 50 were condensed. The isolate which has been sequenced here, was underlined. KGMMV (Kyuri green mottle mosaic virus) included as out-group

Indonesia, Taiwan isolates were classified in the subgroup IA which can be divided into many clades and Iranian isolate fall into distinct clade. Only Pakistan isolate was classified in subgroup IB. In phylogenetic analyses based on ClustalW Multiple alignments, the Iranian isolate in group IA displayed the lowest (88.9%) and the highest (97.9%) nucleotide sequence identity with the isolates Ukraine and W-Japan, respectively. Comparison of the CP amino acid sequence showed exiting the lowest (80 %) and the highest (95.2%) amino acid homology between CGMMV-Kalat and isolates from Ukraine and AL1 (India), respectively (Table 2). In phylogenetic tree based on analysis of the CP amino acid sequences the isolates initially were clustered into two groups and then each group was divided into many subgroups (Fig. 4b).

## DISCUSSION

Many tobamoviruses are distributed worldwide and can infect many field and greenhouse crops (Zhang *et al.*, 2008). Cucurbitaceous crops are an important part of diverse nutrition diet worldwide (Chandra *et al.*, 2010), grown commercially throughout the world, have been destroyed completely locally or epidemically when infected by plant viruses (Yoon *et al.*, 2008). CGMMV causes one of the most common diseases in the family Cucurbitaceae (Slavokhotova *et al.*, 2007).

Results of this study suggest that CGMMV-Kalat isolated from cucumber in Iran has properties common with the genus of *Tobamovirus* (Choi *et al.*, 1998). These include serological relationships, RT-PCR, host ranges and nucleotide sequence of the CP gene. The host range and symptomatology of CGMMV-Kalat were similar in most respects to other CGMMV isolates described for CGMMV-HY1 strain (Shim *et al.*, 2005) and also nearly were similar to Greece isolates (Varveri *et al.*, 2002). The most typical symptoms were observed in greenhouse cucumber with systemic mosaic and varying degrees of mottle mosaic on leaves and this was in agreement with Kim *et al.* (2003). DAS-ELISA is a common diagnostic technique in survey studies to detect plant viruses. However, the RT-PCR technique is highly sensitive, simple and useful in overcoming many difficulties encountered with serological methods. It seems also that RT-PCR is faster than traditional methods and other methods such as dot blot hybridization (Sidaros *et al.*, 2009).

Although all samples showed systemic mosaic and mild to severe yellow mottling symptoms, only 19.7% of samples were infected with CGMMV. This low percentage might be due to the fact that mottle mosaic is a complex disease that can be caused by different viruses such as Cucumber Mosaic Virus (CMV), Watermelon Mosaic Virus-2 (WMV-2), Squash Mosaic Virus (SqMV), Zucchini Yellow Mosaic Virus (ZYMV), as well as by CGMMV (Shabanian *et al.*, 2007) and the symptoms observed may have been caused by one or more of those other viruses.

Phylogenetic analyses were done by the Neighbor-Joining (NJ) method implemented with ClustalW alignment which was used to compare the similarities between the coding nucleotides and their encoded protein. Sequencing results of a Coat portion confirmed the RT-PCR analysis and showed slight variations between the sequence of the CP gene of CGMMV and that of other strains from other parts in the world. The percentage similarities for nucleotide and amino acid between CGMMV-kalat and fourteen isolates clustered in group-I ranged from 88.9 to 97.9% and from 80 to 95.2%, respectively. This confirm that CGMMV-Kalat isolated from cucumber has belonging to genus of *Tobamovirus*.

The study provides clear evidence that CGMMV occurs in Khorasan province. However, from an epidemiological point of view; much work still needs to be done. For example, the source of CGMMV must be investigated and its spread to locations where it does not occur should be prevented. Here, it is important to note that, due to the lack of efficient products for chemical treatment under field conditions, control of diseases caused by viruses is difficult. Consequently, preventive measures to avoid planting of contaminated material are of the highest importance in the context of contaminated material of an integrated approach to control. Among such measures, testing of planting material for pathogen-free status is an important, although not exclusive method for controlling viral disease of plants (Youssef and Shalaby, 2009). Tobamoviruses form very stable particles making them easy to spread and difficult to eliminate. So the best defense against Tobamoviruses is avoidance and the first line of defense is to grow plants with resistance to tobamoviruses. The main danger for the cucumber plants is free virus which is released into the soil after decay of the plant debris (Budzanivska *et al.*, 2006). Virus-free seeds should be used and care should be taken to not infect transplants during production to ensure that only uninfected

transplants are set in the field. As the virus is seed transmitted and easily transmitted mechanically (Hull, 2002) efforts have been made to produce virus-free seeds. Hot water and hot air treatments, using widely varying temperature and treatment times, have demonstrated the most functional methods for inactivating viruses on seeds or plants (Kim and Lee, 2000).

Data shown in this study represent the first report on the characterization of CGMMV at the molecular level in Iran. Previous studies reported the occurrence of the virus (Ghorbani, 1986) but did not provide information about it at the molecular level. Indeed, to our knowledge, this is the first report on the occurrence of CGMMV in cucumber in Iran based on biological reactions, RT-PCR and CP gene analyses. The data obtained in this study will be beneficial to improve control strategies for this virus in Iran. Hence, identification and differentiation of isolates and strains of CGMMV, particularly analysis of its genetic diversity and evolution can be finding procedures for evolution and development of the pathogen and as result presenting the appropriate solution to its control.

In conclusion, we have determined one CGMMV isolate (Kalat) in Iran based on biological reactions, RT-PCR and sequences of CP gene and demonstrated its similarity and phylogenetic relationships, to the other isolates of CGMMV recorded in NCBI. The CP gene of CGMMV-Kalat shared 97.9% identity in nucleotide level and 94.5% in amino acid level with W-Japan isolate.

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