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Detection of Important Viruses on *Dianthus Caryophillus* L. in Markazi Province, Iran, and Molecular Analysis of Carnation Vein Mottle Virus

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ABSTRACT

To investigate the infection of carnations (*Dianthus caryophillus*) to common viruses, a total of 130 samples showing suspected viral disease symptoms were collected from 10 commercial greenhouses located in Mahallat City, Markazi Province. Total DNA and RNA were extracted and amplified by PCR and RT-PCR methods, respectively, using generic primers for the genera *Potyvirus* and *Orthotospovirus* and also specific primers of *Carnation mottle virus* (CarMV), *Carnation necrotic fleck virus* (CNFV), *Carnation etched ring virus* (CERV), *Carnation latent virus* (CLV), *Carnation vein mottle virus* (CVMV) and *Cucumber mosaic virus* (CMV). Results revealed that the estimated rates of infection in the collected samples for different viruses in decreasing order were for CarMV, CNFV, CERV, CMV and CVMV, while CLV and species of genus *Orthotospovirus* were not detected. Mixed viral infections were also investigated and among all of the 130 collected samples, 3.1% showed mixed infections with CarMV, CNFV, CVMV, CERV and CMV, 32% with CarMV, CNFV, and CERV, 20% with CarMV and CNFV, and 9% with CarMV and CERV. For phylogenetical assays, NIb-CP fragment of the CVMV genome was amplified, cloned, and sequenced. Phylogenetic trees of two isolates of this virus revealed that the two Iranian isolates of CVMV (IBR and RCM) were phylogenetically divergent. Mechanical inoculation of CVMV on 16 different indicator plant species showed specific symptoms on six of the indicators that were confirmed by RT-PCR. To the best of our knowledge, this is the first report of CMV and first partial genome sequencing of CVMV from a carnation plant in Iran.

Keywords: Cucumber mosaic virus, Carnation vein mottle virus, Mixed viral infections, Phylogeny.

INTRODUCTION

The genus *Dianthus* L. is one of the most important ornamental genera that contain more than 200 described species. Carnation (*Dianthus caryophillus* L.) is among the top five economically important cut flowers in the world (Lisa, 1995). Mahallat City, Markazi Province, located in central Iran, is one of the greatest producers of cut flowers including carnation, with 57.6 million cut flowers annually (Anonymous, 2019).

Several pests and pathogens affect the production of carnations including viruses. Six plant viruses, namely, Carnation etched ring virus (CERV), Carnation latent virus (CLV), Carnation mottle virus (CarMV), Carnation necrotic fleck virus (CNFV), Carnation ring spot virus (CRSV) and Carnation vein mottle virus (CVMV) have been reported as carnation specific economically important viruses (Hollings

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and Stone, 1970; Lovisolo and Lisa, 1978; Lisa, 1995; Singh *et al*., 2005; Yu *et al*., 2006; Cevik *et al*., 2010). Carnation could also be infected by common plant viruses such as Cucumber mosaic virus (CMV) (Bezić *et al*., 1983; Severin and González, 1996; De La Torre-Almaráz *et al*., 2015). CMV is known as one of the economically important plant viruses worldwide, which belongs to the genus *Cucumovirus* in the family *Bromoviridae*, and classically divided into two different subgroups I and II. Both subgroups are reported from different regions in Iran (Bashir *et al*., 2006; Rasoulpour and Izadpanah, 2008; Nematollahi *et al*., 2012; Shahmohammadi *et al*., 2019). Apart from CRSV, three other specific carnation viruses including CarMV, CERV, and CVMV have been identified serologically from Iran (Bayat, 2009). In addition, *Potyvirus* and *Orthotospovirus* are two economically important viral genera that could infect many ornamental plant species (Rybicki, 2015). The virus is easily transmitted in both genera, which could increase the chance of disease spreading and causing epidemic infections. *Potyvirus* and *Orthotospovirus* species could also be transmitted by aphids and thrips, respectively, although both genera could be easily transmitted by mechanical inoculation (Govier et al., 1977; Goldbach and Peters, 1994).

Studies on the occurrence and distribution of carnation viruses including CERV, CNFV, CarMV and CVMV were carried out by serological and molecular methods in Iran (Safari *et al.*, 2009; Bayat, 2009; Bayat and Ghotbi, 2010). Recently, a molecular heterogenicity of CarMV was reported from two important centers of the cut flowers production in Iran (Barzegar, 2018). Among the carnation viruses studied in Iran, *CVMV* was detected only by serological method (Bayat, 2009; Bayat and Ghotbi, 2010) and no information is available on the molecular data of this virus from Iran. Also, *Orthotospovirus* and *Potyvirus* species have been reported on ornamental plants from Markazi Province (Bayat *et al*., 2018; Ghotbi *et al*., 2005).

Carnation viruses often cause specific or general symptoms on the host or indicator plants. The common symptoms caused by ornamental viruses are faint local chlorotic or necrotic lesions, grayish-white or reddish-purple necrotic flecks, streaks or spots beside mottling, malformation of the leaves, and stunting (Hollings, 1965; Hakkaart, 1972; De La Torre-Almaráz *et al*., 2015). However, in many cases, the virus can cause non-specific symptoms such as mosaic, chlorosis, stunting and necrosis in infected plants (Hakkaart, 1968). It is difficult to define the virus species based on the type of symptoms caused on the main host or their indicator plants. Accordingly, it seems to be essential to use molecular techniques to identify these viruses.

Currently, little is known about the virome of carnation in Mahallat City Commercial Greenhouses, Markazi Province, Iran. Hence, the first aim of this study was the detection of highly important viruses of carnation by PCR-based method in commercial greenhouses of Mahallat. Since no knowledge is available on the phylogeny and genome sequence of Iranian CVMV isolates, the second aim of the present study was determination of phylogenetic affinities of two Iranian CVMV isolates based on NIb-CP partial sequence among other *Potyviridae* members.

MATERIALS AND METHODS

Sampling

From 2015 to 2017, about 130 samples of carnation (*Dianthus caryophillus* L.), including whole plants devoid of roots, were collected from 10 greenhouses located at Mahallat City (Table 1). The collected samples showed symptoms of mottling, purple-white necrotic spots and flecks, reddish-purple discoloration and ring spot on leaves, seedling stunting, and some samples were symptomless.

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Table 1. Time and place of sampling and collected numbers of carnation samples.

Sampling date	Collecting location	No of sample
November 2015	Ornamental plants research center greenhouse	5
	Private greenhouse complex 1	6
	Private greenhouse complex 2	5
	Private greenhouse complex 3	5
	Private greenhouse complex 4	5
February 2016	Ornamental plants research center greenhouse	5
	Private greenhouse complex 1	5
	Private greenhouse complex 2	5
	Private greenhouse complex 3	5
	Private greenhouse complex 4	5
May 2016	Ornamental plants research center greenhouse	6
	Private greenhouse complex 1	5
	Private greenhouse complex 2	5
	Private greenhouse complex 3	5
	Private greenhouse complex 4	5
August 2016	Ornamental plants research center greenhouse	5
	Private greenhouse complex 1	6
	Private greenhouse complex 2	6
	Private greenhouse complex 3	5
	Private greenhouse complex 4	5
November 2016	Ornamental plants research center greenhouse	5
	Private greenhouse complex 1	5
	Private greenhouse complex 2	5
	Private greenhouse complex 3	6
	Private greenhouse complex 4	5
Total		130

DNA/RNA Extraction and PCR

RNA extractions were performed using RNA-Plus extraction kit (Cinnagen, Iran) and the extraction of DNA was done using CTAB method (Stefanova *et al*., 2013) from all specimens. For three viruses, namely, *CarMV*, CVMV and CLV, specific primers were designed based on part of the Coat Protein (CP) gene from known sequences of CarMV (KR002041, X02986), CVMV (KJ605654) and CLV (X52627), and for CNFV, primers were designed based on partial of the CNFV (GU234166) Heat Shock Protein (HSP) gene. *Potyvirus* and *Orthotospovirus* generic primers were used to target the CP and nucleocapsid genes, respectively (Zheng *et al*., 2010, Hassani-Mehraban *et al*., 2016). CERV specific primers to target the partial of Movement Protein (MP) gene were used in PCR (Raikhy *et al*., 2006) and for CMV specific primers of partial CP gene were used (Grieco *et al*., 2000). The oligonucleotides designed and used in this study are presented in Table 2. cDNAs were synthesized in a final volume of 20 µL using 10 µL of RT master mix (Gene All, South Korea), 2 µL of the random hexamer, 2 µL of DEPC water and 6 µL of extracted RNA with a temperature cycle of 42°C for 60 minutes followed by 55°C for 10 minutes. PCR was performed in a final volume of 20 µL containing 10 µM Master RED (Ampliqon, Denmark), 1 µL of each primer (10 picomols), 5 µL of distilled water and 3 µL of DNA (or cDNA) (250 ng μL^{-1}) in the thermocycler (Eppendorf, Germany). Primary denaturing was performed at 94°C for 4 minutes followed by 35 cycles consisting of DNA denaturation at 94°C for 50 seconds, a primer annealing for 50 seconds with a

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specific temperature of each primer (Table 2), an extension at 72°C for 60 seconds and a final fragment extension at 72°C for 10 minutes. In most reactions, *18S rRNA* gene was used as an internal control. PCR products were electrophoresed on a 1% agarose gel in a 1X TAE buffer that was stained by Green Viewer (SMOBIO, Taiwan). The virus species were identified according to the amplified fragments size.

Cloning

In order to clone the partial NIb-CP region of CVMV, the RT-PCR reactions were performed using the primer pair of NIb2-F and CVMV-R according to the above-mentioned program. A fragment consisting of a partial sequence of the NIb and CP of two CVMV isolates was inserted and cloned in the pTG/19 plasmid kit (CinnaClone, Iran) according to the kit instructions. All given PCR products were sequenced by Macrogen Company, South Korea.

Phylogenetic Analysis

The obtained sequences were deposited in GenBank with accession numbers MF068808 and MF068809 for *CVMV* isolates IBR and RCM, respectively. Nucleotide sequences were analyzed by three different methods (bayesian, maximum likelihood, and maximum parsimony) using two different sequence regions including CP, NIb and their combination with 33 other sequences obtained from GenBank (Table 3). The model of base substitution was evaluated using MrModeltest 2 (Nylander, 2004). The Akaike-supported model, a

Table 2. Generic and specific primers designed and used in the current study.

Target	Primers sequence	Annealing temprature (◦C)	Amplicon size (bp)	Reference
Orthotospovirus (Eurasian)	*AS-EA-F: GGGGGATCCAGAGCAATCGAGG* *EA-R: TTGTTCAATGAAGCAGCACC*	52	793	Hassani-Mehraban *et al*. (2016)
Orthotospovirus (Asian)	*AS-EA-F: GGGGGATCCAGAGCAATCGAGG* *AS1-R: GCTTCAGTCCTCTTAAATGTCC*	52	369	Hassani-Mehraban *et al*. (2016)
Orthotospovirus (American)	*AM1-F: GGGGGATCCAGAGCAATTGTGTC* *AM1-R: CTTTGCTTTTCAGCACAGTGCAd*	52	762	Hassani-Mehraban *et al*. (2016)
Potyviruses	*NIb2F: GTITGYGTIGAYGAYTTYAAYAA* *NIb3R: TCIACIACIGTIGAIGGYTGNCC*	45	350	Zheng *et al*. (2010)
CarMV	*F: GAAGAGGAGAGCTGAAATGC* *R: CTTATCGTTGCTTGCCTGTG*	49	645	This study
CVMV	*F: ATCTGTTTAACACCCGCAGC* *R: GAGTGATCACATCCGAACGC*	50	571	This study
CERV	*F: TCCCCCGGGGGAATGAATTCCTCTGTTGA* *R: TCCCCCGGGGGATCATGATATATTGTTTA*	56	980	Raikhy *et al*. (2006)
CNFV	*F: CAGGATTCCAGTTACATACC* *R: ATATCACCTCCAGAACTACG*	46	635	This study
CMV	*F: TAACCTCCCAGTTCTCACCGT* *R: CCATCACCTTAGCTTCCATGT*	51	540	Grieco *et al*. (2000)
CLV	*F: AACTTGCAGCCATAACAGCC* *R: TCCTTACCATACATCCCGCC*	50	511	This study
18S rDNA (internal control)	*F: AACGGCTACCACATCCAAG* *R: AAGCCCTAGCCTCATTACT*	52	459	Faria *et al*. (2006)

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Table 3. The GenBank accession numbers of the virus isolates used in phylogenetic analysis in this study**.**

Virus name	Accession number	Reference
Alstroemeria mosaic virus	AB158522	Fuji *et al.* (2004)
Amaranthus leaf mottle virus	AJ580021	Segundo *et al*. (2007)
Bidens mottle virus	EU250212	Chen *et al*. (2012)
Brugmansia mosaic virus	JX867236	Zhao *et al*. (2013)
Canna Yellow Streak Virus	EF466138	Monger *et al*. (2007)
Carnation vein mottle virus	AY512554	Chung *et al*. (2004)
Carnation vein mottle virus	KJ605654	Unpublished
Carnation vein mottle virus	AB017630	Unpublished
Commelina mild mosaic virus	HQ225836	Zheng *et al*. (2011)
Crinum mosaic virus	KX911718	Unpublished
Freesia mosaic virus	AY685219	Jordan *et al*. (2004)
Hardenbergia mosaic virus	NC015394	Wylie and Jones (2012)
Hippeastrum mosaic virus	GQ857550	Unpublished
Hyacinth mosaic virus	EF203681	Pham *et al*. (2008)
Iris severe mosaic virus	FJ481099	Yan *et al*. (2010)
Lily mottle virus	AB054886	Yamaji *et al*. (2001)
Lupine mosaic virus	NC014898	Sarkisova and Petrzik (2011)
Narcissus degeneration virus	JQ395041	Wylie and Jones (2012)
Narcissus late season yellows virus	JX156421	Wylie *et al*. (2014)
Narcissus yellow stripe virus	JQ395042	Wylie and Jones (2012)
Nerine yellow stripe virus	EF362621	Pham *et al*. (2008)
Ornithogalum mosaic virus	AY994107	Wei *et al*. (2006)
Passiflora virus Y	AB679294	Chiang *et al*. (2012)
Ryegrass mosaic virus	AF035637	Mackenzie *et al*. (1999)
South African passiflora virus	D10053	Brand *et al*. (1993)
Sunflower chlorotic mottle virus	AF255677	Dujovny *et al*. (2000)
Tradescantia mild mosaic virus	AY861351	Ciuffo *et al*. (2006)
Tradescantia mild mosaic virus	GQ847531	Unpublished
Triteleia mosaic virus	GU270649	Miglino *et al*. (2010)
Tulip band-breaking virus	AB078007	Se and Kanematsu (2002)
Turnip mosaic virus	AY995214	Unpublished
Turnip mosaic virus	AF185963	Unpublished
Vallota mosaic virus	FJ618540	Pearson *et al*. (2009)

general time reversible model, including among-site rate heterogeneity and estimates of invariant sites (GTR+I+G), was used in the phylogenetic analyses. Bayesian analysis was performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) running the chains for 2×10^6 generations. For Maximum Likelihood analysis, the same dataset used for the Bayesian tree was employed and the analysis were performed using RAxMl-GUI version 1 for maximum likelihood (Silvestro and Michalak, 2012) and MEGA 4 for maximum parsimony (Tamura *et al*., 2007). *Ryegrass mosaic virus* was selected as an outgroup for all analyses.

CVMV Inoculation to Indicator Plants

To evaluate the host range of CVMV as a newly occurred virus on carnation in Iran, its biological purification was performed by mechanical inoculation of infected carnation plant extract using 0.01 M phosphate buffer on indicator plant *Chenopodium quinoa* followed by the local lesion passage. The purified virus was inoculated into 16 other

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putative indicator plants (Table 4). All inoculations were performed at the 2 to 4 leaf growth stages of indicator plants. All plants were grown in a growth chamber at constant temperature of 22±3°C and 45% relative humidity with 10 hours of light daily. One to 3 weeks after inoculation, symptoms on each indicator plant were recorded. Also, infected indicator plants were tested using RT-PCR with specific primers to confirm the presence of CVMV.

RESULTS

Amplified fragments using PCR and RT-PCR showed an approximately 645 bp length fragment for CarMV, also 635bp for CNFV, 950 bp for CERV, 540 bp for CMV and 570 bp for CVMV (Figure 1). The results showed that 87% (113 out of 130), 62% (80 out of 130), 45% (59 out of 130), 4.6% (6 out of 130), 3.1% (4 out of 130) and 0% (0 out of 130) of surveyed samples were infected with CarMV, CNFV, CERV, CMV, CVMV and CLV, respectively. In addition, no samples were infected with *Orthotospovirus* species. Due to the use of specific primers for each species, virus identification was made by relying on amplified fragment size and confirmed by the indicator plants symptoms for CVMV.

Simultaneous infections of CarMV/CNFV/CERV were observed in 32% of samples, also 20% for CarMV/CNFV, 9% for CarMV/CERV and 3.1% for multiple infections of all five of the detected viruses.

Combined CP and NIb region of two isolates of CVMV, namely, IBR from private greenhouse complex 2 and RCM from Ornamental Plants Research Center (OPRC) greenhouse were cloned (Figure 1-F), sequenced, and after editing, submitted to GenBank under accession numbers MF068808 and MF068809, respectively. Among 1704 studied nucleotides in two isolates, 1,641 nucleotides were identical and 63 were variable between two studied isolates. A Blast-n search revealed the highest similarity of both isolates with three isolates of CVMV (AF017630, KJ605654 and AY512554).

According to the reconstructed phylogenetic trees, and their topology, among nine surveyed trees regenerated by three mentioned methods, the Bayesian tree generated by CP region of CVMV was selected as the best and fittest phylogenetic tree based on the total tree topology (Figure 2). In the Bayes generated phylogenetic tree based on CP region of CVMV, two Iranian

Table 4. Symptoms of *Carnation vein mottle virus* in different indicator plants after mechanical inoculation.

Scientific name	Family	Symptoms
Brassica campestris	Brassicaceae	Not apparent
Chenopodium amaranticolor	Chenopodiaceae	Leaf local chlorosis and necrosis
Chenopodium murale	Chenopodiaceae	Not apparent
Chenopodium quinoa	Chenopodiaceae	Leaf local chlorosis
Cucumis sativus	Cucurbitaceae	Not apparent
Gomphrena globosa	Amaranthaceae	Not apparent
Nicotiana benthamiana	Solanaceae	Leaf chlorosis
Nicotiana glutinosa	Solanaceae	Not apparent
Nicotiana occidentalis	Solanaceae	Mosaic and deformation of leaf
Nicotiana rustica	Solanaceae	Not apparent
Nicotiana tabacum	Solanaceae	Leaf vein clearing
Phaseolus vulgaris	Fabaceae	Not apparent
Physalis alkekengi	Solanaceae	Not apparent
Pisum sativum	Fabaceae	Not apparent
Vicia faba	Fabaceae	Not apparent
Vigna unguiculata	Fabaceae	Leaf mosaic

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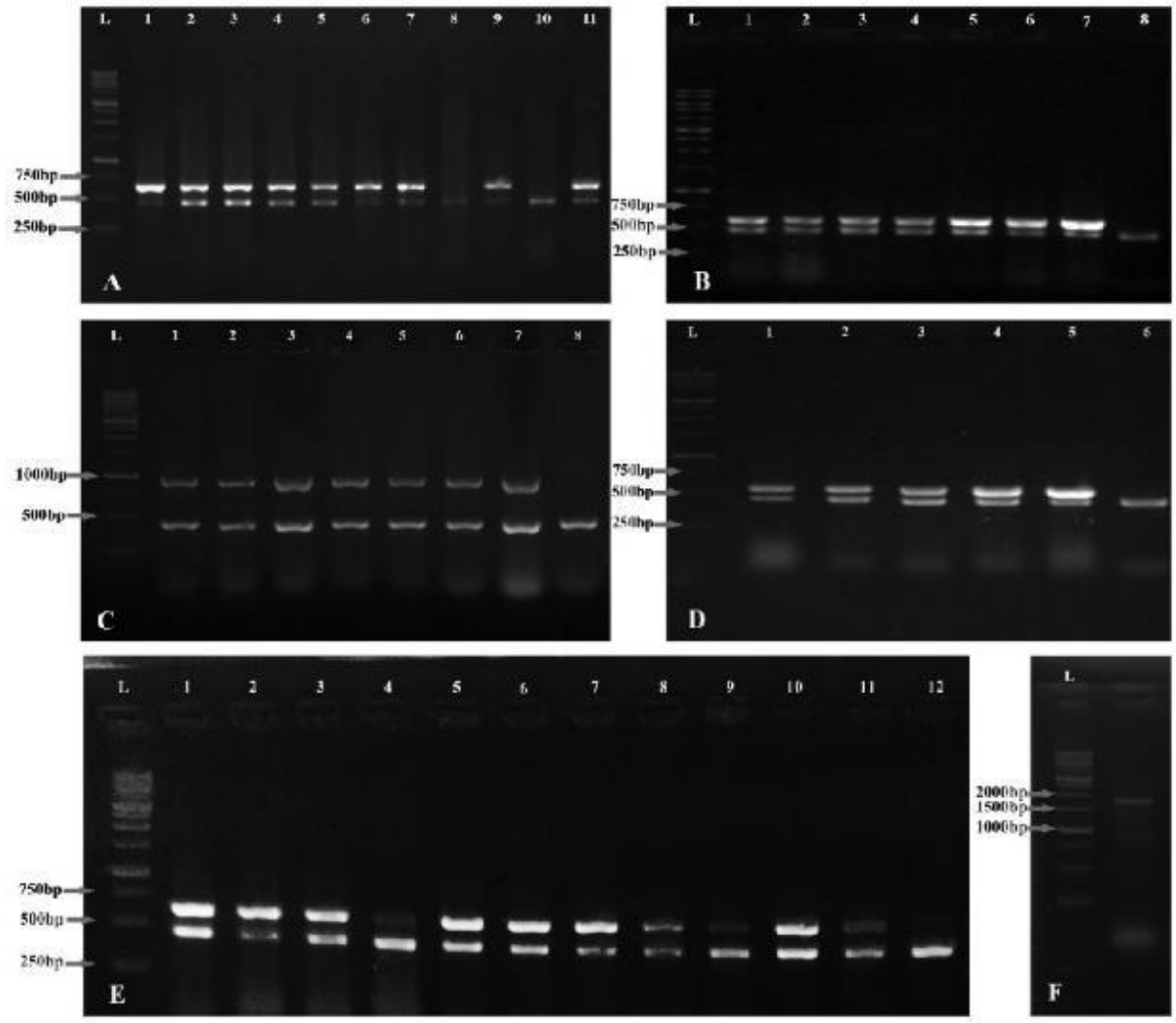

Figure 1. Electrophoresed PCR products on 1% Agarose gel. A: *Carnation mottle virus* (1-7 and 9: Infected plants; 8: Uninfected plant 10: Negative control; 11: Positive control); B: *Cucumber mosaic virus* (1-6: Infected plants; 7: Positive control; 8: Negative control), C: *Carnation etched ring virus* (1-6: Infected plants; 7: Positive control; 8: Negative control); D: *Carnation vein mottle virus* (1-4: Infected plants; 5: Positive control; 6: Negative control); E*: Carnation necrotic fleck virus* (1-10: Infected plants; 11: Positive control; 12: Negative control); F: Cloned and amplified *Carnation vein mottle virus* NIb/CP region using specific primers. In all samples, the 459 bp fragments were presented 18S rRNA as internal control. L: GenRuler™ 1 kb DNA ladder (Fermentas).

isolates (IBR and RCM) from two different samplings, but with the same symptoms on carnation plants, generated a highly supported monophyletic clade with other studied CVMV isolates. The isolate IBR formed a sister group with two isolates CVMV-K and JLCC and RCM isolate had a close affinity with other CVMVs.

To indicate the CVMV host range, the virus was purified biologically and inoculated on other plants (Figure 3, Table 4). The mechanical inoculation of CVMV to *C. amaranticolor* revealed local chlorotic lesions 7-14 days post-inoculation (dpi), which turned to necrotic spots in one week, as reported by Hollings (1965). In *Vigna unguiculata* inoculated with CVMV 10-14 dpi, mosaic was observed on the leaves.

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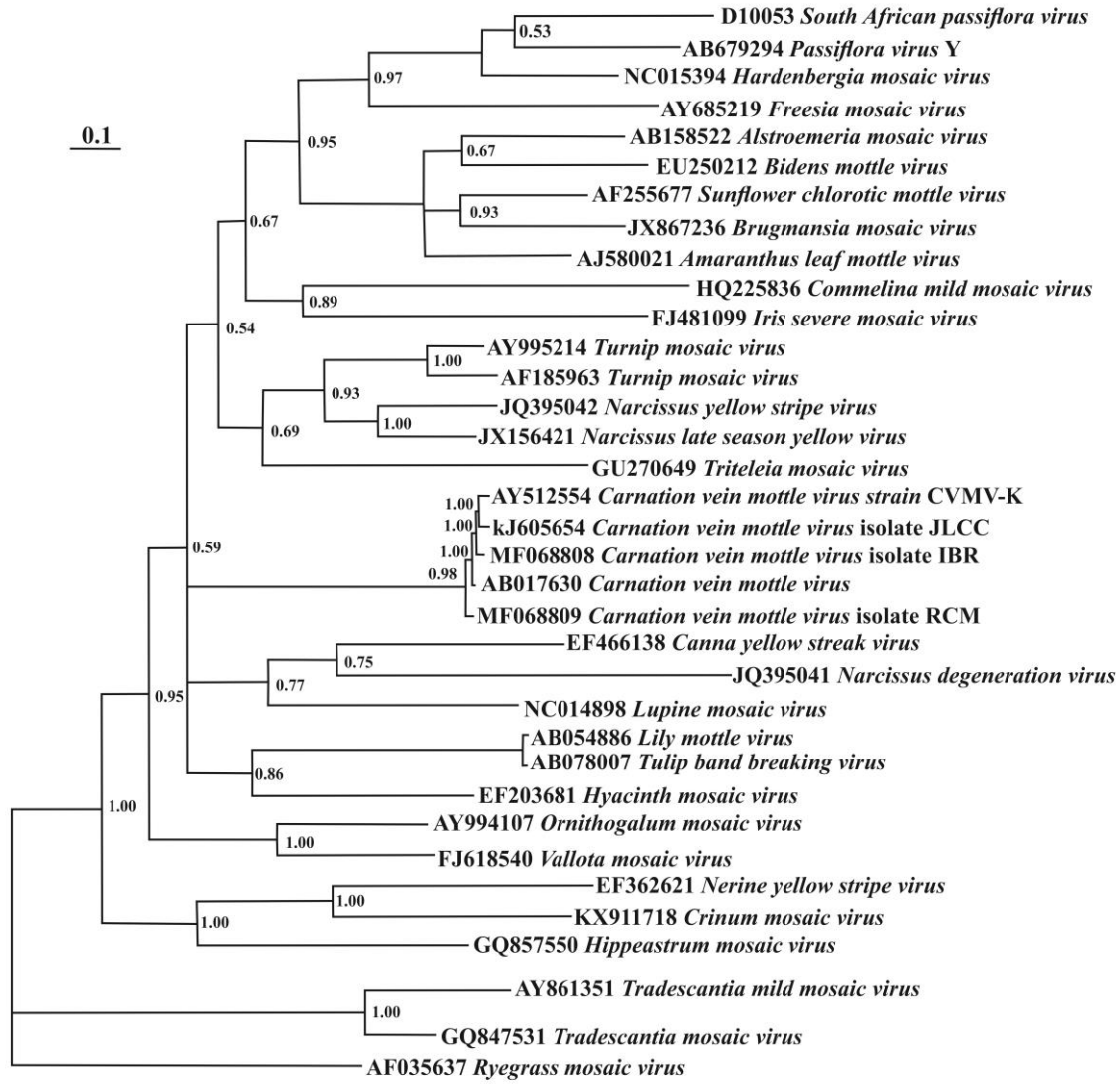

Figure 2. Bayesian tree inferred under the GTR+I+G model from CP region sequences of *Carnation vein mottle virus* (CVMV) and other related viruses generated by MrBayes software. Posterior probability is given on appropriate clades and *Ryegrass mosaic virus* is selected as the out-group.

CVMV inoculation to indicator plants showed chlorosis in *Nicotiana benthamiana* in 8-10 dpi, and a vein clearing in *N. tabacum* in 12-14 dpi. For *N. occidentalis*, in 7 to 12 dpi, mosaic and deformities were observed in the leaves. CVMV inoculation to *C. quinoa* showed local chlorotic leaf spots in 7-10 dpi, which were consistent with a previous study (Hollings and Stone 1970). The occurrence of these symptoms along with the results of the RT-PCR in naturally infected and inoculated plants and sequence identities confirmed the presence of CVMV in Iran.

DISCUSSION

In this study, the virome of carnation in Iran was investigated by PCR and RT-PCR. The results indicated positive reactions for five important carnation viruses including

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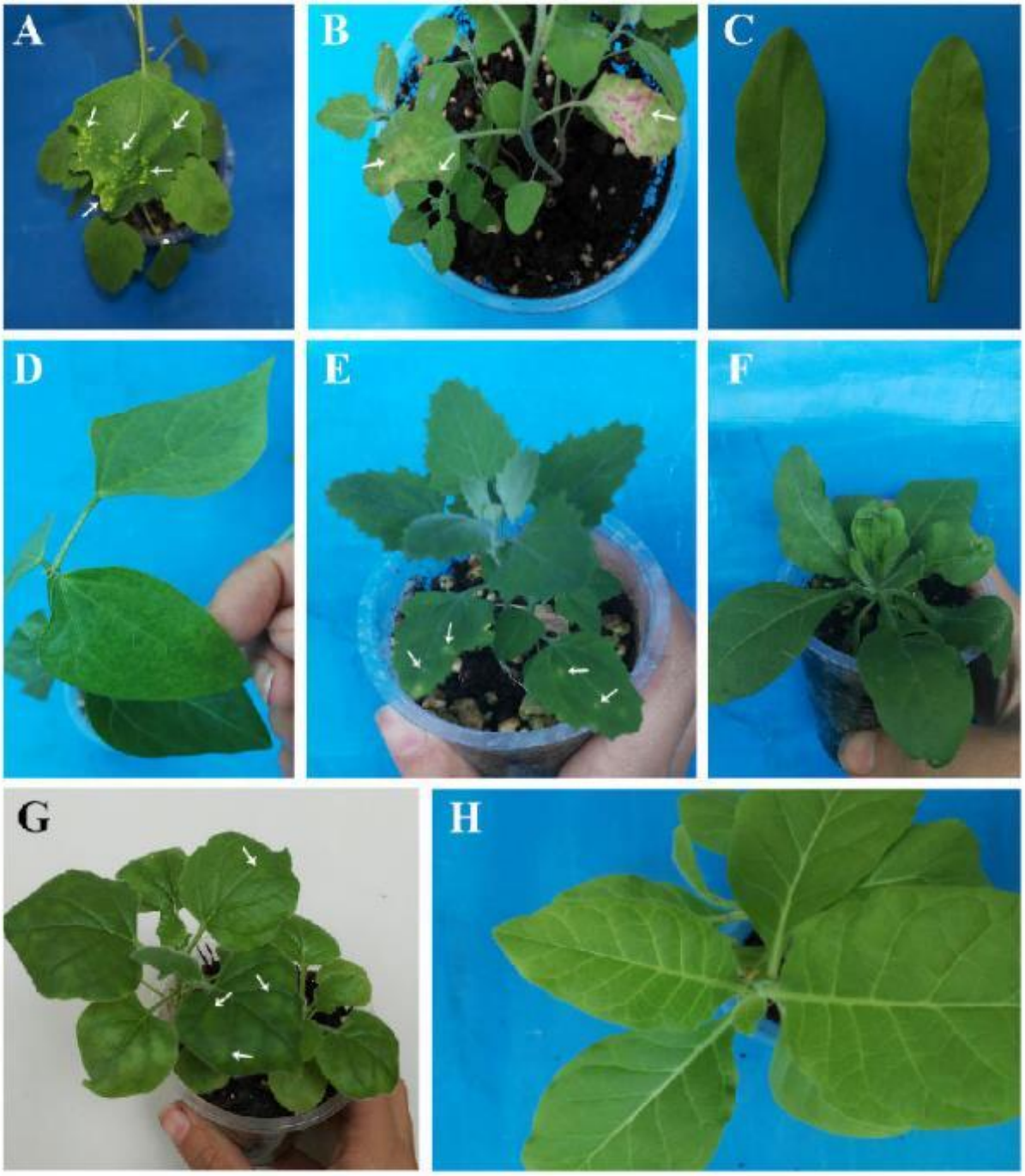

Figure 3. Symptoms on different indicator plants inoculated by CVMV. A and B: *Chenopodium quinoa* (local chlorosis), C and F: *Nicotiana occidentalis* (mosaic symptoms and deformation of leaves), D: *Vigna unguiculata* (leaf mosaic symptoms), E: *C. amaranticolor* (leaf local chlorosis and necrosis), G: *N. benthamiana* (leaf local chlorosis), H: *N. tabacum* (vein clearing).

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CarMV, CNFV, CERV, CMV, and CVMV with 87%, 62%, 45%, 4.6%, and 3.1%, respectively, among 130 surveyed samples in Mahallat City Commercial Greenhouses. No *Orthotospovirus* species and CLV were detected from the collected samples. A similar study was conducted in California using Enzyme-Linked Immunosorbent Assay (ELISA) for some carnation specific viruses, namely, CarMV, CNFV, CRSV and CERV on commercial curnation cultivars. Among 226 tested samples, 78, 13 and 15% of samples were infected to CarMV, CNFV and CERV, respectively, and no samples were infected with the CRSV. Among the analyzed samples, 21% were infected by two or more viruses (Lommel *et al*., 1983). In Mexico, RT-PCR and ELISA were used to detect carnation viruses. Two viruses including CarMV, and CNFV were detected, also, simultaneous infection of these two viruses has been reported (De La Torre-Almaráz *et al*., 2015).

Previous studies in Iran showed that all currently detected viruses were investigated by serological or molecular methods. Bayat and Ghotbi (2010) showed that CVMV could be traced by serological method from carnation plants; the same virus has been detected by molecular method in the current study. Also, Bayat (2009) by serological method showed that the carnation plants could be infected to CERV and CNFV. Safari *et al.* (2009) showed that CarMV could be detected by DAS-ELISA in carnation tissues. Barzegar (2018) detected CarMV by molecular methods. These findings confirm that CarMV, CNFV, and *CERV* are widespread in Iran and the world and thus emphasizes the need for indexing and production of virus-free quality planting materials.

Mixed infections with different viruses have been reported in carnation plant over a wide geographic area. In the present study, multiple viral infections of carnation were also investigated in Mahallat City Commercial Greenhouses. The results showed that simultaneous infections of five carnation viruses including CarMV, CNFV, CERV, CMV and CVMV occurred in 3.1% of surveyed samples, mixed infection of CarMV/CNFV/CERV was observed in 32% of samples, also 20% for CarMV/CNFV, and 9% for CarMV/CERV. In Korea, RT-PCR and ELISA have revealed the occurrence of two carnation viruses, CNFV and CERV, occurring simultaneously (Lee *et al*., 2013). The simultaneous infection of CarMV/CNFV/CERV, CarMV/CNFV and CarMV/CERV was recorded in previous studies (Lommel *et al*., 1983). Although the mixed virus infection has been reported in carnation, to the best of our knowledge, this is the first report of the simultaneous infections for five carnation viruses in Iran.

In this study, the incidence of CMV in carnation plants was investigated. Although CMV has been reported in diverse plants in Iran (Sokhandan *et al*., 2008; Hassanvand and Shams-bakhsh, 2017; Siampour and Izadpanah, 2020), this is the first report of CMV infected carnations from Iran (Figure 1-B). Carnation infection to CMV has also been reported from several countries. In Yugoslavia, carnation infection with CMV has been detected using agar gel double-diffusion assay and electron microscopy, and the results showed that the plants were simultaneously infected with CarMV and CMV (Bezić *et al*., 1983). Also, in another study conducted in Mexico, using RT-PCR and Dot-blot method, CMV was detected in carnation, where plants were simultaneously infected with CarMV, CERV and CMV (De La Torre-Almaráz *et al*., 2015).

There are some earlier publications on CVMV as an economically important virus of carnation. Raikhy *et al*., 2004 detected the virus from India using ELISA. Also, the virus was detected using the same method from China (Li *et al*., 2014) and South Korea (Chung *et al*., 2004). There has been a good deal of study on *CVMV* elimination in plants due to its economic importance to carnation (Ahmed *et al*., 2012). Therefore, the molecular characterization of *CVMV* as a rapid technique is an important approach to avoid virus spreading. The current paper is focusing on the molecular properties of the

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virus. In the Bayes generated phylogenetic tree based on the CP region of CVMV, two Iranian isolates from two different sampling areas, but with the same symptoms on plants, formed a highly supported monophyletic clade with other studied CVMV isolates. The isolate IBR formed a sister group with two isolates CVMV-K and JLCC and RCM isolate has a close affinity with other CVMVs. In all regenerated trees, the two Iranian CVMV isolates (IBR and RCM) had genetic divergence, which could indicate that they were imported from different counties or regions to Iran. The Bayesian tree generated by the CP region of CVMV is supported by Adams *et al*. (2005) who proposed a phylogenetic analysis of the fully-sequenced species among the family *Potyviridae* and suggested the CP region as the fittest fragment for phylogenetic analysis. To our knowledge, this is the first report of the genome sequence of the Iranian CVMV isolates.

Carnation is an economically important cut flower and its viruses has been studied widely. Understanding the incidence and distribution of these viruses, along with the development of a rapid, sensitive and accurate detection method for these viruses, would be a great help to prevent and control virus damages.

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ردیابی ویروس‌های مهم *Dianthus Caryophillus* L. در استان مرکزی و آنالیز مولکولی ویروس پیسک رگبرگ میخک

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چکیده

به منظور بررسی آلودگی گیاه میخک به ویروس‌های بیماری‌زای رایج، در مجموع ۱۳۰ نمونه مشکوک به آلودگی ویروسی از ۱۶ گلخانه تجاری شهرستان محلات جمع آوری شد. نمونه‌ها پس از بررسی علائم و استخراج ماده ژنتیکی به روش PCR و RT-PCR توسط آغازگرهای اختصاصی جنس‌های *Orthotospovirus* و *Potyvirus* و گونه‌های carnation mottle virus (CarMV)، carnation necrotic fleck virus (CNFV)، Carnation etched ring

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carnation vein (CVMV)، carnation latent virus (CLV)، virus (CERV) mottle virus و cucumber mosaic virus (CMV) مورد بررسی قرار گرفتند. در نتیجه مشخص گردید که از کل نمونه های جمع آوری شده بیشترین درصد آلودگی به ترتیب مربوط به CarMV، CNFV، CERV، CVMV و CMV بود. نتایج نشان داد که در بین ۱۳۰ نمونه جمع - آوری شده، حدود ۳ درصد آلودگی پنج‌گانه به CarMV، CNFV، CVMV، CERV و CMV، ۳۲ درصد نمونه‌ها آلودگی سه‌گانه به ویروس‌های CarMV و CNFV و CERV، ۲۰ درصد آلودگی دوگانه به CarMV و CNFV و ۹ درصد آلودگی دوگانه به CarMV و CERV داشتند. دو گونه CLV و اعضای جنس *Orthotospovirus* در این تحقیق ردیابی نشدند. برای مطالعه ملکولی CVMV قطعه ای شامل NIb-CP ویروس تکثیر و توالی یابی شد. تجزیه و تحلیل تبارشناسی دو جدایه از این ویروس نشان داد که جدایه‌های ایرانی با حمایت بالا از سایر جدایه ها متمایز هستند. همچنین مایه زنی مکانیکی CVMV روی ۱۶ گیاه محک متفاوت سبب بروز علائم اختصاصی روی شش گیاه محک شد که با آزمون RT-PCRتایید شد. بر اساس اطلاعات موجود، این اولین گزارش از وقوع CMV در گیاه میخک از ایران و همچنین ردیابی ملکولی و تعیین توالی بخشی از ژنوم جدایه‌های ایرانی CVMV است.

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