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Prevalence and genetic diversity of fig mosaic virus isolates infecting fig tree in Iran

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Summary. – Commercial and outdoor fig orchards in four Iranian provinces were surveyed for the incidence of fig mosaic virus (FMV), fig leaf mottle associated virus 2 (FLMaV-2) and fig mild mottle associated virus (FMMaV) from March 2011 to October 2012. A total of 350 asymptomatic and symptomatic fig samples were collected and tested by dot-immunobinding assay (DIBA) for the fig mosaic disease (FMD) using a polyclonal antiserum. According to DIBA results, FMD was present in 73% of the collected symptomatic samples from all visited regions. Samples with positive reactions in DIBA were then analyzed by RT-PCR using with specific primers. PCR results showed that about 14.8% of the FMD-positive samples from three inspected provinces are infected with at least one virus. FMV was the most widely spread virus (14%) followed by FLMaV-2 (1.5%), whereas FMMaV was not found. Phylogenetic analysis of the glycoprotein nucleotide and amino acid sequences of known FMV isolates showed two independent groups with high bootstrap values, with all Iranian isolates distinctly clustered in group I, subgroup IA beside those reported in Turkey. Nucleotide diversity was high within but low between different selected geographic regions and except for Europe, nucleotide distance within geographic regions was low. Statistical analyses indicated a correlation between the genetic structure of the FMV isolates and the geographical origin of isolation. Our analyses suggested that the FMV population is in a state of increase following a bottleneck or founder event in Iran.

Keywords: Ficus carica; phylogeny; genetic diversity; RT-PCR; sequencing

Introduction

Cultivated fig (*Ficus carica* L.), an important tree plant of the family *Moraceae*, is one of the oldest domesticated crops in Iran and the world. Most of the fig cultivars have arisen by the process of traditional selection in Iran. Fig tree was supposed to have entered into Iran and other Asian countries form the East Mediterranean countries like Turkey, where its genetic resources are still available as wild fig, and is considered to be the origin of this plant (Condit, 1955). Fig mosaic disease is the most common virus disease of fig plants. The etiology of FMD is still under investigation, but it was found that it is caused by one or more viruses from a complex of several viruses infecting fig trees in different fig growing countries. This complex comprises 16 different viruses belonging to different genera including: *Bunyaviridae*, *Caulimoviridae*, *Closteroviridae*, *Flexiviridae*, *Partitiviridae*, and *Tymoviridae* (Elbeaino *et al*., 2006, 2007a, 2009, 2010, 2011a,b; Gattoni *et al*., 2009).

FMD causes many disorders in fig trees, leading to poor fruit quality, reduction of productivity, shortening of the tree life and it affects the tree growth of many commercial cultivars (Walia *et al*., 2009; Martelli, 2011). Little is known about the etiology of FMD and its transmis-

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Abbreviations: DIBA = dot-immunobinding assay; FLMaV-2 = fig leaf mottle associated virus 2; FMD = fig mosaic disease; FMMaV = fig mild mottle associated virus; FMV = fig mosaic virus; HSP70 = heat shock-protein 70; Gp = glycoprotein; TSWV = tomato spotted wilt virus

sibility (Caglayan *et al.*, 2010). Fig mosaic virus (FMV) belongs to the recently established the genus *Emaravirus* of the family *Bunyaviridae* (Adams and Carstens, 2012). It is a six partite negative-sense RNA virus that is recognised as the main viral agent involved in the fig mosaic disease (Elbeaino *et al.*, 2012). Like other emaraviruses, the genome of FMV contains RNAs with monocistronic ORF, each of which codes for a single protein (Elbeaino *et al.*, 2012). Genomic ssRNAs range in size from 7.09 kb to 1.212 kb (Elbeaino *et al.*, 2012; Walia and Falk, 2012). During 2006–2010, the incidence of FMD increased in the western and southern provinces of Iran, causing significant yield losses. The cause of the sudden emergence of FMD was undetermined until our previous research that showed the existence of FLV-1, FLMaV-1, and FMV associated with the mosaic-affected fig trees throughout the gardens in the mentioned regions of Iran (Shahmirzaie *et al.*, 2012). The objective of the present study was to provide new data about existing FMV strains and their genetic diversity in the most important fig plantations in central, eastern and northern regions of Iran.

Materials and Methods

Sample collection and field observations. Samples were collected from commercial and outdoor fig gardens in 4 provinces of Iran, including Alburz (in northern part of Iran), Khorasan-Razavi (In east), Mazandaran (In north) and Tehran (in the centre), from March 2011 to October 2012 (Table 1). In total, 66 gardens in twenty two cities were randomly surveyed and 350 samples were collected from symptomatic (256 samples) and asymptomatic (94 samples) leaves. A sample consisted of two young leaves per plant. DIBA test was done with the universal polyclonal antibodies (Istituto Agronomico Mediterraneo di Bari, Via Ceglie 9, 70010 Valenzano (BA), Italy) against viruses that are involved in FMD. Samples with positive reactions in DIBA were then inspected by RT-PCR for the presence of FLMaV-2, FMV and FMMaV. Healthy fig plants obtained by tissue culture were used as negative controls.

RNA isolation and RT-PCR. Fig samples that positively reacted with the specific polyclonal antibody against FMD in DIBA and proved to be infected with fig mosaic viral complex were further subjected to RT-PCR. Using the GF-TR-100 RNA Isolation mini Kit (Vivantis, Malaysia), total ribonucleic acid was extracted from

Table 1. Incidence of FMV and FLMaV-2 infections in symptomatic fig samples collected from fig orchards in Iran

Province	Region	Tested trees	Infected trees[a]		FMV		FMMaV		FLMaV-2	
		No	No	%	No	%	No	%	No	%
Alburz (0)[b]	Bilaghan	4	0	0	0	0	0	0	0	0
	Fardis	34	0	0	0	0	0	0	0	0
	Hashtgerd	12	0	0	0	0	0	0	0	0
	khozancola	2	0	0	0	0	0	0	0	0
	Mahdasht	8	0	0	0	0	0	0	0	0
	Porkan	6	0	0	0	0	0	0	0	0
	Zibadasht	8	0	0	0	0	0	0	0	0
Khorasan- Razavi (14.8)	Bardeskan	8	2	25	2	25	0	0	2	25
	Ghochan	2	0	0	0	0	0	0	0	0
	Kashmar	4	0	0	0	0	0	0	0	0
	Mashhad	28	4	14.3	4	14.3	0	0	0	0
	Neshaboor	4	0	0	0	0	0	0	0	0
	Shandiz	4	0	0	0	0	0	0	0	0
	Torghabeh	4	2	50	2	[c]**50**	0	0	0	0
Mazandaran (25)	Amol	10	2	20	0	0	0	0	2	20
	Babolsar	2	0	0	0	0	0	0	0	0
	Baijan	8	4	50	4	**50**	0	0	0	0
	Fereidon Kenar	10	3	30	3	30	0	0	0	0
	Ramsar	10	1	10	1	10	0	0	0	0
Tehran (22.7)	Islamshahr	8	0	0	0	0	0	0	0	0
	Tehran	58	17	29.3	17	**29.3**	0	0	0	0
	Varamin	22	3	13.6	3	13.6	0	0	0	0
Total Infection		256	38		36		0	0	4	
Mean Infection				14.8		14		0		1.5

[a]Data obtained by RT-PCR for FMV. [b]Percentage of FMV infection in each province. [c]The highest incidence rate of FMV in each surveyed province is shown in bold.

100 mg of fig leaf tissues according to the manufacturer's instructions. First-strand cDNA synthesis was carried out using M-MuLV reverse transcriptase (MBI, Fermentas, Germany) according to the manufacturer's instructions in a 20µl reaction mixture. For the detection of FLMaV-2, FMMaV, and FMV three sets of virus-specific primers were used in RT-PCR (Table 3). The PCR was carried out with 2.5 µl cDNA in a total volume of 25 µl containing 1.5 mmol/l $MgCl_2$, 0.2 mmol/l dNTPs, 0.3 µmol/l of each primer, and 1unit of *Taq* DNA polymerase (Cinnagen, Iran). Amplifications were carried out in a thermocycler (Eppendorf, Hamburg, Germany) using an initial denaturation at 94°C for 4 min, followed by 35 cycles at 94°C for 30 sec, 50 to 55°C for 35 sec, 72°C for 45 sec, and a final incubation at 72°C for 7 min. Primers were synthesized by MWG Biotech (Germany). Amplification products were analyzed by electrophoresis in 1% agarose gel containing ethidium bromide and visualized under UV light. Positive and negative samples were kindly supplied by Dr. Toufic Elbeaino (Bari, Italy).

Sequencing and phylogenetic analysis. The amplified PCR fragments were subjected to purification with the Ultra-Clean purification kit as described by the manufacturer (Fermentas Gene JET TM Gel Extraction Kit, Germany) and cloned into the pTZ57R/T vector according to the manufacturer's instructions (MBI Fermentas, Germany). *Escherichia coli* DH5α were made competent according to Chung *et al.* (1989) and transformed with the ligation mix. Recombinant plasmids were sequenced using an ABI PRISMcc 3730XL automated sequencer (Macrogen Inc., Seoul, South Korea) and the sequence was deposited in GenBank (http://www.ncbi.nlm.nih.gov/ BLAST/). At least two clones of each selected isolate were sequenced. The GenBank Acc. Nos. of these sequences are listed in Table 2. For subsequent procedures, six FMV and the only detected

Table 2. Values from pairwise sequence comparisons based on BLAST analysis for heat shock-protein 70 (HSP70) and glycoprotein (Gp) genomic regions amongst FLMaV-2 and FMV isolates, respectively. Geographic origin and GenBank Acc. Nos. of FMV isolates used for sequence comparisons in this study

Virus	Acc. No.	Isolate	Origin	Nucleotide (nt) and amino acid (aa) sequences identity with reference*	
				nt	aa
FMV	AB697828	JS1	Japan	96	99
FMV	AB697829	SB1	Serbia	90	100
FMV	FM864225	GR10	Italy	100	100
FMV	FJ211074	JJW	USA	97	99
FMV	GU227409	EI-D1-3	Turkey	90	99
FMV	GU227413	EI-BS-15	Turkey	94	99
FMV	GU227415	D2	Turkey	93	99
FMV	HQ703344	CAN01	Canada	92	99
FMV	JQ173768	Morguz1Gp2	Turkey	92	98
FMV	JQ173770	SariZeybek3Gp4	Turkey	84	96
FMV	JQ173784	NoiredeCoromb2Gp18	Turkey	92	96
FMV	JQ173799	Sarilop1MKUCOGp33	Turkey	93	97
FMV	KC408932	EMV- 1	Iran	89	100
FMV	KC408933	EMV- 3	Iran	89	95
FMV	KC408934	EMV- 4	Iran	88	98
FMV	KC408935	EMF- S3	Iran	86	99
FMV	KC408936	EMF- S9	Iran	88	99
FMV	KC408937	FE-10	Iran	89	99
FLMaV-2	FJ473383	F3	Italy	100	100
FLMaV-2	FN666271	L2I	Turkey	95	99
FLMaV-2	FN668733	IDB	Turkey	96	99
FLMaV-2	FN687736	Alb12	Albania	99	99
FLMaV-2	FN687737	ALg3	Algeria	99	99
FLMaV-2	FN687738	LiB 53	Lebanon	96	99
FLMaV-2	FN687743	LiB 80	Lebanon	94	99
FLMaV-2	FN687745	Syr 8	Syria	98	99
FLMaV-2	FN687747	Tu58	Tunisia	92	99
FLMaV-2	KC534878	M2	Iran	93	99
FMV[a]	KC261957	TSWV- 7	South Korea		

*Italian isolates GR10 (FM864225.2) and F3 (FJ473383) were used as reference isolates for FMV and FLMaV-2, respectively. [a]Tomato spotted wilt virus (TSWV) members of the genus *Tospovirus* used as an outgroup species.

FLMaV-2, isolates were selected based on the geographical origin of the samples. Phylogenetic analysis of the selected Iranian isolates was conducted by comparing separately the 450bp nucleotides (nt) of the partial Gp gene of FMV with the comparable sequences of other corresponding isolates from GenBank using the BLAST (NCBI) program (Altschul *et al*., 1997).

Statistical tests of genetic differentiation, gene flow and estimation of genetic distance. Phylogenetic analyses were done by the neighbor-joining methods using MEGA 5.05 under the assumption of the substitution models proposed. The phylogenetic tree was constructed using the neighbor-joining algorithm (Saitou and Nei, 1987), p-distance method (Nei and Kumar, 2000) and bootstrap consisting of 1000 pseudo-replicates, and finally evaluated using the interior branch test method with Mega 5.05 software (Tamura *et al*., 2011). The nucleotide sequences were also translated to amino acid sequences and analyzed using the neighbor-joining method with Mega 5.05 program. Multiple alignment analysis was done by CLUSTAL W (Thompson *et al*., 1994). The nucleotide distance (average genetic distance between two randomly selected genetic variants) between pairs of sequences was estimated by the same software using the Kimura 2-parameter method (Kimura, 1980). The total populations of FMV isolates were divided into subpopulations based on different sampling regions. Within and between populations, diversities were estimated according to Nei (Nei, 1987) based on the T92+G model. The Tajima's statistic (Tajima, 1989) was also calculated to test the mutation neutrality hypothesis. Statistics as well as the natural selection pressure were calculated by the DnaSP 5.10 program (Librado and Rozas, 2009).

Results

According to DIBA results, FMD has occurred in collected symptomatic samples (73%) from four provinces surveyed, including Alburz, Khorasan-Razavi, Mazandaran and Tehran. The incidences of FMD detected by DIBA differed between the surveyed provinces. FMD symptoms of mosaic restricted with a necrosis border, mottling and leaf malformations were observed in the surveyed fields. Due to mixed infections, it was difficult to identify the symptoms of a particular virus in the garden.

RT-PCR was done using as template symptomatic samples from infected fig trees (256 samples), which positively reacted with the anti-FMD antiserum in the DIBA test. Infection of the Iranian fig trees with FLMaV-2 and FMV, but not FMMaV, was confirmed by RT-PCR. PCR amplification of the FLMaV-2 and FMV genome fragments with the primers specific for the HSP70 and glycoprotein genes yielded the expected 360 bp and *ca*. 468bp amplification products. No PCR products were amplified from healthy plants.

Of 256 samples with positive reaction to FMD antiserum in DIBA assay, 38 (14.8%) contained plants infected by at least one of detected viruses when tested by RT-PCR. According to RT-PCR results, FMV was the prevailing virus (14%), followed by FLMaV-2 (1.5%). FLMaV-2 and FMV relative incidence varied among the geographical regions they were found. FLMaV-2 was found in the eastern and northern regions, whereas FMV was detected in the central, northern and eastern parts of Iran. Mazandaran province had the highest prevalence of infection (25%), followed by Tehran (22.7%) and Khorasan-Razavi (14.8%) districts. Among the viruses, FMV was detected most frequently in visited regions, it was found in samples collected from Baijan, Bardeskan, Fereidon Kenar, Mashhad, Ramsar, Tehran, Torghabeh and Varamin districts with infection rates of 10% to 50% (Table 1). Highest incidence of FMV was found in Tehran province (22.7%) (Table 1). FLMaV-2 was detected in Bardeskan and Amol districts with infection rates of 25 and 20%, respectively. It was detected in mixed infection with FMV in 0.8% of the collected samples. FLMMaV was not detected in any of tested samples.

The sequences reported in this paper were submitted to GenBank under the Acc. Nos. KC408932-KC408937 for FMV and KC534878 for FLMaV-2. Accessions related to the different provinces, where the viruses occurred in Iran, include: Mazandaran (KC408935, KC408936, and KC534878); Mashhad (KC408933 and KC408934) and Tehran (KC408932 and KC408937). Pairwise nucleotide comparison of Iranian FMV isolates showed low levels of divergence amongst them. On the basis of the BLAST analysis and sequence alignments, the nucleotide (nt) and amino acid (aa) sequence identities of the Gp gene of six Iranian FMV isolates ranged from 90 to 100% and 95 to 100%, respectively. Obtained Iranian FMV sequences were related to the reference FMV nucleotide sequences (Acc. No. FM864225.2) with more than 86% and 95% identities at the nucleotide and amino acid levels, re-

Table 3. List of primers used in RT-PCR for fig viruses detection

Viruses	Primers	Primer sequences (5′-3′)	Amplified product (bp)	Target gene region	Reference
FLMaV-2	F3-s	GAACAGTGCCTATCAGTTTGATTTG	360	HSP70	Elbeaino *et al.* (2007)
	F3-a	TCCCACCTCCTGCGAAGCTAGAGAA			
FMMaV	LM3-s	AAGGGGAATCTACAAGGGTCG	311	HSP70	Elbeaino *et al.* (2010)
	LM3-a	TATTACGCGCTTGAGGATTGC			
FMV	EMARV-GP-s	GGGTACATATGCGTCATTCTTG	470	Gp	Walia *et al.* (2009)
	EMARV-GP-a	CGTTTGTCTTGGATCACAGCAA			

spectively (Table 2). BLAST analysis of one FLMaV-2 isolate from Iran (KC534878) disclosed 92–100% and 99–100% identities at the nucleotide and amino acid levels with nine other sequences for the HSP70 genomic region from the GenBank (Table 2).

Phylogenetic tree based on the Gp nucleotide sequence alignments showed that all the FMV isolates are closely related, but formed two distinctive groups, in which each group is divided into two subgroups (Fig. 1). The six Iranian representative FMV isolates were shown to belong to separate subgroups (a and b) in group I (Figs. 1a and b). Iranian isolates stood beside Turkish isolates (EI-D1-3 and EI-BS-15) in Subgroup IA. Global isolates including FMV isolates collected from different countries (Canada, Italy, Japan, Serbia, Turkey and USA) were categorized in subgroup IB. Geographical clustering was observed for Iranian isolates in subgroup IA, in which isolates EMF-S3 and EMF-S9 from Mazandaran province in the north of Iran were clustered separately (Fig. 1a).

The tree reconstructed from amino acid sequences clustered the FMV isolates into two main groups and two subgroups in Group I on the basis of the Gp genomic region (Fig. 1b). Sequences from Iran were distributed between subgroups A and B in group I and segregated according to the geographical origin. Isolates in subgroup IA (EF-10, EMF-S3, EMV-1, EMV-3, and EMV-4) originated from the central (Tehran), northern (Mazandaran) and eastern (Mashhad) parts of the country, whereas isolates in subgroup IB (EMF-S9) was detected in the northern part of the country (Mazandaran) (Fig. 1b). To analyze whether selected FMV isolates from different geographical origins are genetically differentiated populations, statistical tests of population were done. The FMV populations were divided into three geographic subpopulations: 2 isolates from America (Canada and USA), 10 isolates from Asia (Iran, Japan and isolates reported from the southern regions of Turkey) and 6 isolates from Europe (Italy, Serbia and isolates detected from western regions of Turkey). Nucleotide distances of FMV within Asia, America and Europe were 0.099, 0.089 and 0.236, respectively. Distance between subpopulations (continents) was about 0.038 for isolates from the Americas and Asia, 0.045 between America and Europe and 0.062 between Europe and Asia. Nucleotide diversity was high within each subpopulation (a value of 0.125). The Tajima's D was calculated to test the mutation neutrality hypothesis. The statistics was significantly negative, giving a value of – 2.0788 ($P < 0.05$), suggesting a strong negative or purifying selection.

Discussion

This study showed the natural incidence and occurrence of FLMaV-2 and FMV in commercial and outdoor fig gardens in three provinces located in the central, northern and western regions of Iran. FLMaV-2 has been reported to infect fig trees in other countries throughout Africa, Asia,

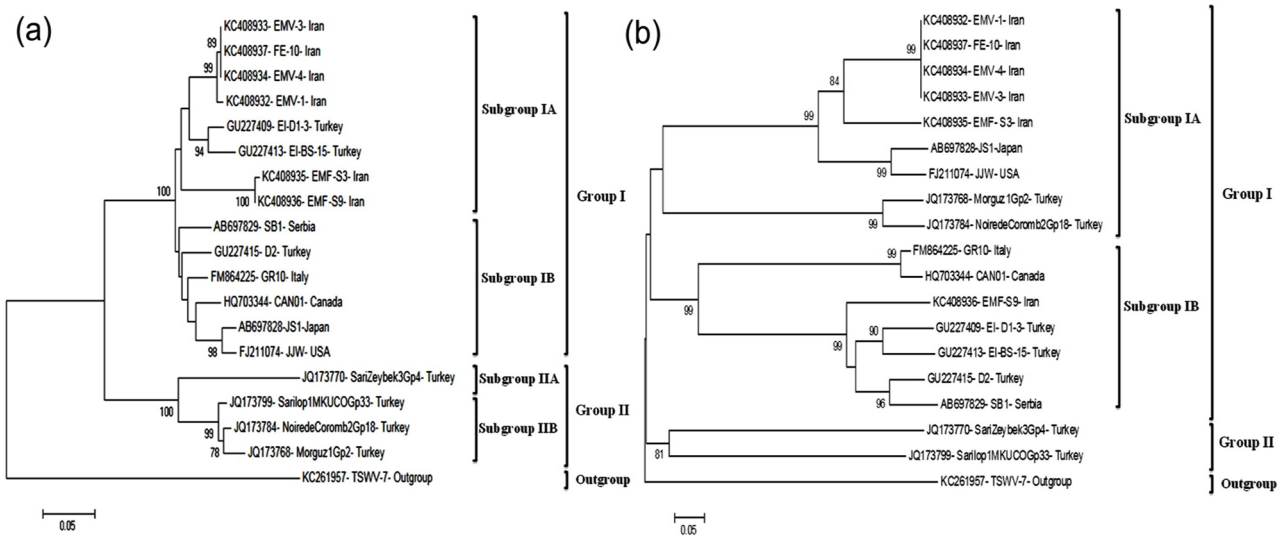

Fig. 1

Phylogenetic trees calculated from the nucleotide sequences (a) and amino acid sequences (b) of Gp-encoding region of fig mosaic virus by the neighbor-joining algorithm, based on calculations derived from the multiple alignment

Numbers at each node indicate bootstrap percentages based on 1000 replications. Only values >50% are shown. The horizontal branch lengths are proportional to the genetic distance. The corresponding sequence of tomato spotted wilt virus (TSWV) was used as outgroup.

and Europe (Elbeaino *et al.*, 2007a,b; Caglar *et al.*, 2011; Elbeshehy and Elbeaino, 2011). The detection of FLMaV-2 in mixed infection with FMV confirms the association of this virus in the etiology of FMD worldwide. Low levels of mixed infections with FLMaV-2 and FMV detected in Mazandaran (0.7%) and Khorasan-Razavi (0.7%) in this study is not a new finding. Similar mixed infections were also observed in fig trees in Turkey (Caglar *et al.*, 2011). It was interesting that the incidence of FMV was considerably high (14%) compared with the remaining tested viruses. It is possible that the FMV inoculum sources, such as infected fig propagative materials, were highly available to spread the virus in the visited regions. As the results above indicate, it can be concluded that FMV was the key virus affecting fig orchards in Iran. Therefore, efforts must be directed towards the control of the disease. The finding that FMV was predominant at sampling sites in Iran is in line with previous information about the cosmopolitan distribution of this virus (Caglar *et al.*, 2011; Elbeaino *et al.*, 2011c; Elbeshehy and Elbeaino, 2011; Martelli, 2011; Elci *et al.*, 2012).

In this study, symptomatic fig samples from all surveyed provinces positively reacted with the universal polyclonal antibody against FMD by DIBA test, in which 14.8% of them proved to be infected with the FLMaV-2 or FMV by RT-PCR. Tested viruses were not recovered from symptomatic samples with FMD infection from Alburz province, indicating the presence of other viral agents involved in the FMD etiology in Iran. This may suggest that FMV does not play a significant role in the etiology of FMD in Iran. However, FMV has been recognised as main causal agent of fig mosaic disease (FMD) worldwide (Elbeaino *et al.*, 2012). Whether very divergent strains of tested viruses are present in the country or other new viruses from different taxonomical groups are responsible for symptom expression in fig trees in the visited regions remains to be determined. In a recent study, fig fleck-associated virus (FFkaV) was reported in symptomatic fig samples from Alburz province (Nouri-Ale Agha *et al.*, 2013). FMMaV was not detected in any of the tested fig samples collected from the central, eastern and northern regions of Iran in this study. Previous study has also shown that this virus is not present in the southern and western regions of Iran, suggesting that this virus is not involved in FMD in Iran (Shahmirzaie *et al.*, 2012). This is in contrast with African, European and Mediterranean countries, where FMMaV was detected with the infection rates of 13.3 to 43.3% (Elbeaino *et al.*, 2007a, 2010, 2011c, 2012; Elbeshehy and Elbeaino, 2011c; Elci *et al.*, 2012).

In this study, we further analyzed the distribution and genetic variation of new FMV isolates in Iran with the aim to further our understanding of its population genetics and phylogenetic relationships. The phylogenetic relationship of all FMV isolates based on nucleotide sequence of the Gp gene showed segregation into two groups, in which each group divided into two distinct subgroups. Branching pattern based on differences in the geographical origin was found among the Iranian FMV isolates in group A, in which isolates EMF-S3 and EMF-S9 from north of Iran were separately clustered. Segregation according to the geographical origin on the basis of the amino acid sequences was also observed. Our conclusion is consistent with previous research on Tomato spotted wilt virus (TSWV), the other member of the family *Bunyaviridae,* showing that TSWV populations display geographical structuring attributed to founder effects (Tsompana *et al.*, 2005). Our study of the Gp gene of 18 isolates indicates the possible existence of variants or strains in the FMV populations and there is at least one major population of FMV in different geographical regions of Iran. The relatively high diversity within each continent (0.125) suggests that the virus might have been long dispersed and accumulating its mutants for a considerable time in each continent. The fact that diversity between continents (0.056) is a little lower than diversity within continents suggests FMV population differentiation by the founder effect and a limited virus movement between continents. Except for Europe, which showed a 0.235 nucleotide distance, nucleotide distance was low within each studied geographic subpopulation, including Asia (0.099) and America (0.089), suggesting a spatial genetic stability among the FMV isolates in Asia and America and the existence of divergent isolates in Europe. The phylogenetic analysis also showed that the Turkish isolates were scattered in all phylogenetic groups and subgroups. The existence of divergent isolates in Europe as well as in Turkey, and the low divergence among the isolates from the rest of the world, suggested that European countries, especially Turkey, could be the origin of FMV and that this genotype might have disseminated worldwide. The phylogenetic analysis generated using the amino acid and nucleotide sequences of the FMV Gp gene showed that Iranian isolates are mostly similar to strains from Turkey, indicating the possible introduction of strains from Turkey into Iran. This is probably due to the exchange of infected propagative plant material. Tajima′s *D* test is based on the differences between the number of segregating sites and the average number of nucleotide differences. We estimated nucleotide polymorphisms in the FMV-Gp populations for each geographical group using their Gp nucleotide sequences by Tajima's D test. Tajima's D test results proved that Gp is evolving neutrally in Iran and worldwide. Negative values also indicated that the FMV population is in a state of increase following a bottleneck or founder event in Iran and that a decrease of the genetic variation by elimination of deleterious mutations by purifying selection maintained the population fitness. The glycoprotein is known to play an important role in maturation and assembly of virion in both plants and the vector by the attachment to receptors in the vector (Bandla *et al.*, 1998). FMV population expansions may be attributed to the

selection constraints, imposed to the Gp sequence by the vector, host plant or the environmental conditions that can be the main causes of the FMV variability observed in Iran. The study of the FMV population demography and genetic diversity lays the foundation for the development of strategies for the control of fig mosaic disease.

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