www.czasopisma.pan.pl PAN POLSKA AKADEMIA NAUK www.journals.pan.pl

JOURNAL OF PLANT PROTECTION RESEARCH Vol. 56, No. 2 (2016)

DOI: 10.1515/jppr-2016-0019

Prevalence and phylogenetic analysis of *Fig mosaic virus* and *Fig badnavirus-1* in Iran

Mohammadreza Alimoradian[1], *Farshad Rakhshandehroo*[1]*, *Masoud Shams-bakhsh*[2]

[1] Department of Plant Pathology, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, 14515-775, Iran

[2] Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, 14115-111, Iran

Received: September 14, 2015
Accepted: April 18, 2016

Abstract: *Fig mosaic virus* (FMV) and *Fig badnavirus-1* (FBV-1) are two of the most important fig infecting viruses. The incidence and distribution of FBV-1 and FMV were determined by testing in PCR 138 asymptomatic and symptomatic samples. These samples were collected from 60 fig gardens and agricultural fields in three provinces of Iran. The fig infecting viruses FBV-1 and FMV, respectively, were detected in 92 (66.6%) and 34 (24.6%) samples collected from all the surveyed fields. Overall, 24 out of 138 (17.3%) samples showed mixed infections. The sequence analysis of a genomic fragment of 922 nt, comprising the entire ORF-2 and part of the 5′ termini of the ORF-3 of 10 selected FBV-1 Iranian isolates from different provinces, and of the type member from GenBank (Acc. No: JF411989), showed a variation ranging from 1 to 3% at nucleotide level and 1% at the amino acid level. The phylogenetic analysis grouped the FBV-1 isolates into two groups, with the Iranian isolates clustered in two distinct subgroups of group I, according to their geographical origin. In our research, the prevalence and sequence analysis of FBV-1 as the only identified DNA virus infecting fig trees, was studied for the first time in Iran.

Key words: FBV-1, FMV, RT-PCR, management, phylogeny, sequencing

Introduction

Because of its impact and prevalence on fig trees, fig mosaic (FMD) is considered one of the most important diseases of fig worldwide. *Fig mosaic virus* (FMV) is considered to be mainly responsible for FMD, although its etiology is not completely known (Condit and Horne 1933; Martelli 2011). *Fig mosaic virus* is an approved species of the genus *Emaravirus* that is spread by fig propagating material and by the viruliferous eriophyid mite *Aceria ficus*, from fig to fig trees (Flock and Wallace 1955), but not by seeds. Its genome comprises six single-stranded negative-sense RNAs (Walia *et al*. 2014). Several other viruses have been repeatedly associated with FMD. To this list, a DNA virus of the genus *Badnavirus* has also been recently added (Laney *et al*. 2012; Minafra *et al*. 2012). Badnaviruses affect a wide range of tropical and subtropical plant species and are mostly transmitted by mealybugs. Badnaviruses includes plant and animal infecting viruses and replicate their genomic DNA via an RNA intermediate using reverse transcription (Medberry *et al.* 1990; Bouhida *et al*. 1993). Like other members of pararetroviruses, their double-stranded DNA genome of about 7–8 kb exists as both episomal and endogenous sequences in the host plant genome (Staginnus *et al*. 2009). DNA of some badnaviruses integrates in the host's chromosome through a process of illegitimate recombination, and the release of activated endogenous viral DNA giving episomal virus from the integrant (Gayral *et al*. 2008; Liu *et al*. 2012; Iskara-Caruana *et al*. 2014). Integration of pararetroviruses genome into the host's chromosomes has been proved for many crop plants such as tobacco, banana, bitter orange, petunia, rice, potato, tomato, Dahlia, pineapple, grapes, and fig (Chabannes and Iskra-Caruana 2013). It has been shown that stress events, such as tissue culture, micropropagation, and nutrition may induce pararetroviruses episomal infections (Dallot *et al*. 2001; Chabannes and Iskra-Caruana 2013). The non-segmented and circular double-stranded DNA genome of *Fig badnavirus-1* (FBV-1) isolates has been completely sequenced and its presence in mosaic-symptomatic and asymptomatic fig trees has been studied (Laney *et al*. 2012). It was found, that FBV-1 is the first virus of the family Caulimoviridae to be detected in fig trees. There is not much information available on the molecular variability of this virus. The population genetics and evolutionary history explaining the presence and distribution of infectious FBV-1 isolates in fig trees and the phylogeny of global isolates are also poorly documented. Investigation into the genetic variability and evolutionary virus mechanisms, such as mutation, recombination, natural selection, genetic drift, and gene flow (Grenfell *et al.* 2004; Acosta-Leal *et al*. 2011) may contribute in designing improved disease management strategies.

*Corresponding address:
rakhshandehroo_fa@srbiau.ac.ir

In the present study, our first aim was to investigate the prevalence of the FBV-1 and FMV, as new viral agents infecting fig trees in Iran. Our second aim was to analyse the phylogenetic relationships of FBV-1 isolates collected from diverse locations.

Materials and Methods

Field survey and plant material

From April to September 2013 and 2014, a total of 138 asymptomatic (60) and symptomatic (78) fig samples were collected from fig trees with typical symptoms of fig mosaic disease (FMD). Disease symptoms included leaf chlorotic and necrotic ringspot, leaf deformation, mosaic, and line patterns. Samples were collected from the following three provinces (and specified districts) of Iran: Alburz (Asara, Karaj, Mahdasht, Savejbolagh), Hamedan (Hamedan, Malayer, Nahavand, Razan), and Mazandaran (Amol, Babol, Behshahr, Nour, Qaem Shahr and Sari) (Table 1). The samples were from the most common local varieties: San Pedro, Sabz, and Zard from 60 different commercial orchards and gardens were at a distance of 15–20 km from each other. All samples were subjected to laboratory tests for the detection of FBV-1 and FMV.

Nucleic acid extraction, reverse transcription-polymerase chain reaction (RT-PCR), PCR and sequencing

Total DNA and RNA extractions were performed according to the protocols described by Dellaporta *et al*. (1983) and Foissac *et al*. (2001), respectively. Total DNAs and RNAs were isolated from leaves of asymptomatic and symptomatic fig plants and the healthy controls. For RNA extraction, 100 mg of leaves were homogenised in extraction buffer containing 1 ml of grinding buffer [4.0 M guanidine thiocyanate, 0.2 M NaOAc pH 5.2, 25 mM EDTA, 1.0 M KOAc, and 2.5% (w/v) PVP-40], and were silica-purified (Foissac *et al*. 2001).

For RT-PCR, the extracted RNA was subjected to reverse transcription using random hexamer primers according to the manufacturer's instructions (MBI, Fermentas, Germany). Subsequent PCR reaction was carried out with specific primer, which was designed on the glycoprotein (Gp) sequence by Walia *et al*. (2009), in 25 µl volume of 2.5 µl of cDNA, 1.5 mM $MgCl_2$, 0.16 mM each of dNTP, 0.3 µM of each primer, and 2.5 U of *Taq* DNA polymerase (Cinnagene, Iran) in the buffer recommended by the manufacturer. Virus-specific primers Badna-P1s/Badna-P1as, designed on the movement protein nucleotide sequence (Minafra *et al*. 2012) were used for the FBV-1 detection (Table 2).

Table 1. Incidence of *Fig badnavirus-1* (FBV-1) and *Fig mosaic virus* (FMV) infections in fig samples collected from fig orchards in Iran

Province	Region	Tested trees no.	Infected trees[a]		FBV-1		FMV	
			no.	%	no.	%	no.	%
Alburz (65.6%)[b]	Asara	22	14	63.6	14	63.6	3	13.6
	Karaj	12	9	75.0	7	58.3	4	**33.3**[c]
	Mahdasht	12	5	41.6	5	41.6	0	0.0
	Savejbolagh	18	14	77.7	14	77.7	1	5.5
						(62.5)[e]		(12.5)
Hamedan (67.6%)	Hamedan	7	4	57.1	4	57.1	3	42.8
	Malayer	9	2	22.2	2	22.2	2	22.2
	Nahavand	12	12	100.0	9	75.0	5	41.6
	Razan	6	5	83.3	4	66.7	2	33.3
						(55.8)		(35.2)
Mazandaran (85%)	Amol	5	2	40.0	2	40.0	2	40.0
	Babol	6	6	100.0	6	100.0	4	66.6
	Behshahr	13	12	92.3	11	84.6	5	38.4
	Qaem Shahr	5	5	100.0	5	100.0	1	20.0
	Nour	2	1	50.0	1	50.0	0	0
	Sari	9	8	88.8	8	89.0	2	22.2
						(82.5)		(35)
Total infection		138	99	–	92	–	34	–
The mean infection				71.7[d]		66.6		24.6

[a]data obtained by PCR and RT-PCR for tested viruses
[b]percentage of FBV-1 and FMV infections in each province
[c]the highest incidence for FBV-1 or FMV infection in each surveyed province is shown in bold
[d]average percentage of FBV-1 and FMV infection for all provinces
[e]incidence of each virus in the surveyed provinces

Table 2. Nucleotide sequences of the *Fig badnavirus-1* (FBV-1) and *Fig mosaic virus* (FMV) viruses-specific primers used in PCR and RT-PCR assays

Virus	Primer names	Primer sequence 5′-3′	Genome position	Amplified fragment [bp]	Reference
FBV-1	Badna-P1s	GCTGATCACAAGAGGCATGA	1732-1751	214	Minafra *et al.* 2012
	Badna-P1as	TCCTTGTTTCCACGTTCCTT	1927-1946		
	Diversity-580F	AGGCTCTAAGGTTAACTGAAG	560-580	1,090	Laney *et al.* 2012
	Diversity-1650R	ATCATCATCGTGTCAGGTATC	1630-1650		
FMV	EMARAV-s	CGTTTGTCTTGGATCACAGCAA	1670-1688	468	Elbeaino *et al.* 2009
	EMARAV-as	GGGTACATATGCGTCATTCTTG	2117-2138		

PCR thermal cycles for FMV and FBV-1 were as follows: 4 min at 94°C followed by 35 cycles at 94°C for 45 s, 50 to 55°C for 50 s, 72°C for 45 s, and a final extension step at 72°C for 5 min. The amplified fragments were excised from agarose gel and purified with the Gene JET TM Gel Extraction Kit (Fermentas, Germany). Then, they were directly subjected to dideoxyterminator cycle sequencing by Macrogen (Seoul, South Korea) and deposited in the GenBank database (the accession numbers are shown in Table 3). At least two clones of amplified sequence of each FBV-1 isolate were selected and sequenced.

For the sequence analysis of ten selected Iranian FBV-1 isolates, the specific primers 580F/1650R, were used, which amplified a 1,090-nucleotide (nt) fragment of the virus genome encompassing the entire ORF-2 coding sequence plus sequences of the 5′ termini of the ORF-3 (Table 2). The obtained sequences were compared with the sequences available in the GenBank database using BLAST (Altschul *et al*. 1997) (Table 3).

Phylogenetic and nucleotide sequence analyses of selected Iranian FBV-1 isolates

Ten FBV-1 isolates including 2A, 4A, 9A, 18A, from Alburz (northern Iran), 2M, 4M, 8M, 14M, from Mazandaran (northern Iran), and Malayer and Razan from Hamedan (western Iran) were sequenced. Phylogenetic analysis was conducted by comparing the 1,090 nt of the FBV-1 genome of the selected Iranian isolates with comparable available sequences of other isolates from GenBank. The sequences were aligned by CLUSTALX 1.8 (Pearson and Lipman 1988) algorithm and multiple sequence alignments of nucleotide sequences used for the analysis of variability. The phylogenetic trees were constructed using the neighbor-joining method (Saitou and Nei 1987), p-distance method (Nei and Kumar 2000). Bootstrap consisted of 1,000 pseudo-replicates and were evaluated using the interior branch test method with MEGA 5.05 software (Tamura *et al*. 2011).

Results

The incidence of symptomatic fig trees ranged from a minimum of 18.47% in Alburz Province to a maximum of 55.45% in Mazandaran Province. Throughout the visited gardens in the different provinces, plant samples that were infected by FBV-1, mostly exhibited leaf deformation. Fig trees that tested positive for both FBV-1 and FMV infection by PCR, exhibited leaf chlorotic and necrotic ringspots.

Incidence of FBV-1 and FMV

Of 138 fig samples tested by PCR for the presence of FBV-1 and FMV, there were 99 (71.7%) infected by at least one virus. The highest prevalence of viruses was found in Mazandaran (85%) (Table 1). The incidence of FBV-1 and FMV varied according to the provinces and districts. It was found that FBV-1 and FMV were respectively detected, in 92 (66.6%) and 34 (24.6%) samples collected from all the surveyed fields. FBV-1 infection rates in the visited provinces in decreasing order were: 82.5% in Mazandaran, 62.5% in Alburz, and 55.8% in Hamedan. This virus was particularly spread in the Babol and Qaem Shahr districts of Mazandaran Province where the totality of tested plants was infected (Table 1). *Fig mosaic virus* was more prevalent in Hamedan (35.2%), followed by Mazandaran (35%), and Alburz (12.5%) provinces, with the peak incidence of infection in Babol district (66.6%). Unlike FBV-1, FMV was not detected in the Mahdasht and Nour districts. Overall, 24 out of 138 (17.3%) samples had mixed infections, ranging from 3.6% in Alburz to 8% in Hamedan.

PCR and phylogenetic analysis

Expected PCR amplicons of 214 bp and 1090 bp were obtained from FBV-1 infected fig samples using Badna-P1s/Badna-P1as and 580F/1650R primers, respectively. Whereas, 470 bp products were obtained from FMV infected samples using the specific primers designed on the glycoprotein gene. No amplification was obtained in any of the healthy plants used as the controls. The obtained sequences were deposited in GenBank under accession numbers KM610208 to KM610217 (Table 3). Accessions related to the FBV-1 isolates occurred in different provinces of Iran including: Alburz (KM610208, KM610209, KM610215, KM610217); Hamedan (KM610212, KM610213), and Mazandaran (KM610210, KM610211, KM610214, KM610216). The use of BLAST analysis disclosed 98–100% identity among the Iranian FBV-1 isolates at the nucleotide level, and 97–100% with the member type (GenBank Accession No. JF411989) (Table 3). Minimum nucleotide sequence identities (96%) were observed between the Iranian (9A, 2M, 4M) and American (OH1) isolates (data not shown).

Table 3. Geographic origin, GenBank accession numbers, and values from pairwise sequence comparisons based on BLAST analysis for the 1 kb of the *Fig badnavirus-1* (FBV-1) genome

Accession no.	Isolate	Origin	Nucleotide (nt) and amino acid (Aa) sequences' identity with reference* [%]	
			nt	Aa
JF411989	Arkansas 1	USA	100	100
JN112365	AR2	USA	99	100
JN112366	AR3	USA	99	100
JN112368	AR5	USA	99	100
JN050859	CA11	USA	99	100
JN050864	CA16	USA	99	100
JN050877	CA32	USA	99	100
JN050882	CA37	USA	99	100
JQ282675	CA41	USA	99	100
JQ282673	MI1	USA	99	100
JQ282668	OH1	USA	99	100
JQ282669	OR1	USA	99	100
JQ282672	SC1	USA	99	100
KM610208	4A	Iran	99	100
KM610209	18A	Iran	99	100
KM610210	2M	Iran	99	100
KM610211	8M	Iran	99	100
KM610212	Razan	Iran	97	99
KM610213	Malayer	Iran	97	99
KM610214	4M	Iran	99	100
KM610215	9A	Iran	99	100
KM610216	14M	Iran	99	100
KM610217	2A	Iran	98	99
FJ560944[a]	Huachano 1	Peru	–	–

*American isolate Arkansas 1 (JF411989) was used as a reference isolates for FBV-1

[a]S*weet potato badnavirus B* (SPBV-B) members of the genus *Badnavirus* used as an out-group species

Along with Iranian FBV-1 isolates, the sequences of the other thirteen FBV-1 isolates of American origin downloaded from GenBank were also considered for the phylogenetic analysis (Table 3). This analysis revealed the presence of two distinctive groups of isolates (Fig. 1). Almost all isolates were distributed in group I, while the sole American OH1 isolate formed the distinct group II. Group I can be further separated into two subgroups (I and II). Isolates from Iran were distributed in both subgroups: isolates in subgroup I originated mostly from the northern (Mazandaran Province) parts of the country, whereas isolates in subgroup II were from the northern and western parts (the Alburz and Hamedan Provinces) of the country (Fig. 1). Distribution of FBV-1 isolates from Alburz into two subgroups may indicate the presence of Alburz strains in different populations.

Discussion

In this research, the prevalence and sequence analysis of the recently identified FBV-1 infecting fig trees was studied. Our previous surveys on fig orchards from different regions of Iran showed that several viruses belonging to different genera, including Fig fleck-associated virus (FFkaV), Fig cryptic virus (FCV), Fig latent virus 1 (FLV-1), Fig leaf mottle-associated virus 1 (FLMaV-1), Fig leaf mottle-associated virus 2 (FLMaV-2), Fig leaf mottle-associated virus 3 (FLMaV-3), and FMV were present in Iranian fig trees with different infection rates. Of these viruses, FFkaV and FLV-1 were the most prevalent ones (Shahmirzaie *et al*. 2012; Nouri Ale-Agha and Rakhshandehroo 2014; Danesh-Amuz *et al*. 2014; Norozian *et al*. 2014). The results of this study have shown that FBV-1 is the prevailing virus in Iranian fig orchards, with an in-

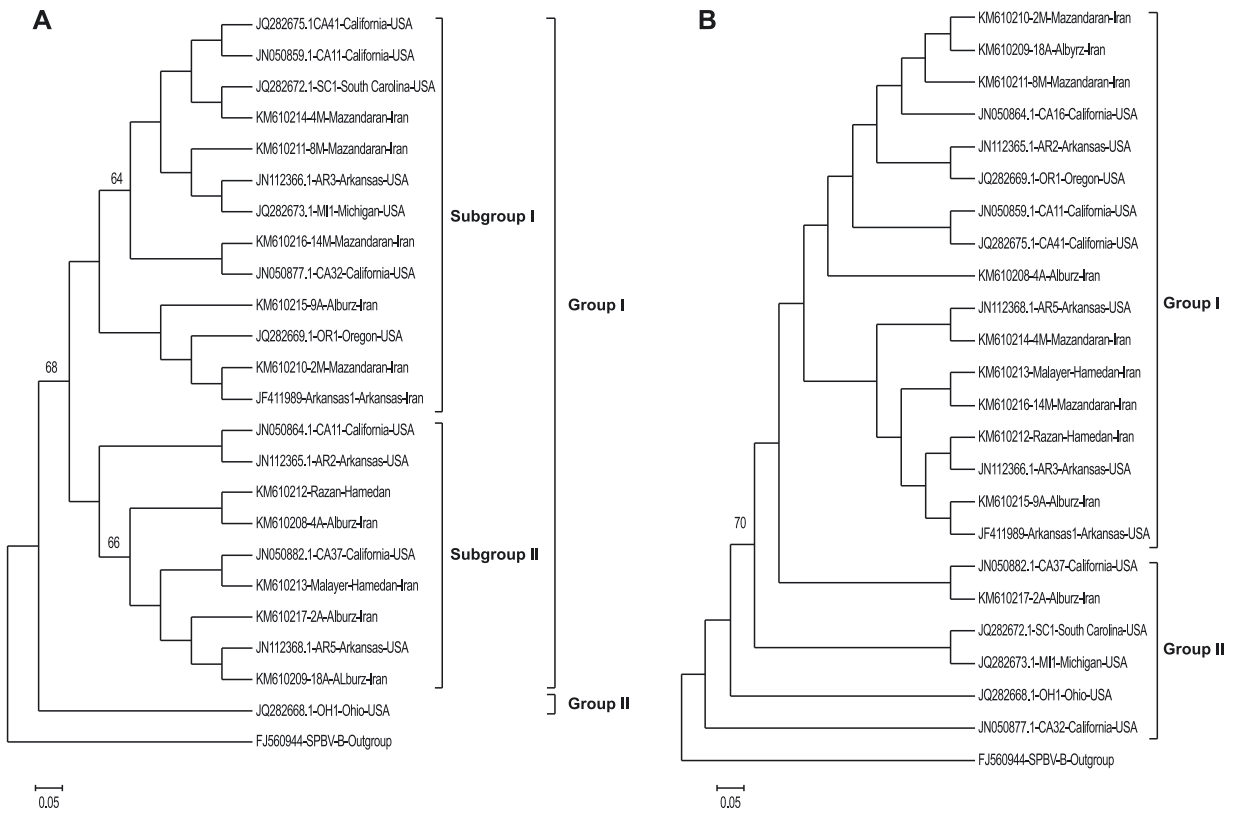

Fig. 1. Phylogram generated from the alignment of (A) nucleotide and (B) amino acid sequences of ten Iranian FBV-1 isolates, together with the homologue gene of those from USA (Table 3) using neighbor-joining algorithm, the p-distance method, and bootstrap consisting of 1,000 pseudoreplicates. Nucleotide sequences of Sweet potato badnavirus B was used as an outgroup to root the tree (Table 3). Only bootstrap values greater than 68% are shown. Branch lengths represent bootstrap values. The bar represents 0.05 changes per site. Bootstrap values less that 65% are not shown as they are considered unreliable

fection rate of 66.6%; about three times more than FMV (24.6%) (Table 1). In our study, FBV-1 was detected in orchards from all the provinces surveyed. The presence of an inoculum source, susceptible hosts, and vectors could have accounted for the uneven and high distribution of FBV-1. The incidence of FBV-1 (66.6%) was considerably high if compared with FMV and other viruses previously recorded on fig trees in Iran and other countries (Elbeaino *et al*. 2011a, b, c; Elbeshehy and Elbeaino 2011; Elbeaino *et al*. 2012; Nouri Ale-Agha and Rakhshandehroo 2013; Danesh-Amuz *et al*. 2014). Most of the surveyed regions showed the highest level of prevalence of FBV-1. This finding may suggest that FBV-1 has the highest economic impact on fig trees compared to other known viruses. However, FBV-1 incidence in Iran can be considered low if compared with the results obtained in the United States (97.4%), Europe (87 to 100%), and Mediterranean countries (100%) (Elci *et al*. 2012; Laney *et al*. 2012; Minafra *et al*. 2012). The high global incidence of FBV-1 suggests that the virus may be efficiently transmitted in figs in nature. It has also been recently reported that FBV-1 genome can integrate into fig chromosomal DNA (Laney *et al*. 2012). In the study by Laney *et al*. (2012), it was noted that FBV-1 can also be readily transmitted mechanically to herbaceous plants. Altogether, vegetative propagation is of major importance for virus spread. On the other hand, badnaviruses can be potentially transmitted through seeds (Hearon and Locke 1984; Martin and Kim 1987), pollen (Hearon and Locke 1984), and mealybugs (Meyer *et al*. 2008), which might have maximised FBV-1 dispersal between countries. This means that care needs to be taken to avoid the use of FBV-1 infected parents in fig breeding programs, and disease control must be based on the use of virus-free stock plants for propagation.

The incidence of FBV-1 and FMV infections in the northern regions of Iran (Alburz and Mazandaran) were significantly higher than those in the Hamedan Province (Table 1). The incidence of FBV-1 ranged from 40 to 100%, while that of FMV ranged from 0 to 66.6% (Table 1). The high incidence of FMV in the Mazandaran Province may reflect a very high inoculum of this virus in the northern regions of Iran.

Mixed infection of FBV-1 with FMV was detected in 17.3% of the samples. The detection of FBV-1 in the mixed infection with FMV confirms the association of this virus in the etiology of FMD in Iran. The same high frequency of mixed infection between FBV-1 and FMV, was previously reported in fig growing fields in Turkey (Elci *et al*. 2012). Mixed infections with badnaviruses are common in nature and they can induce synergistic interactions with other viruses (Laney *et al*. 2012). More studies are needed to determine the possible effect of FBV-1 in mixed infection, on the etiology of fig mosaic disease.

Previous studies showed a significant correlation between genetic variation and pathogenicity of DNA viruses (Oluwafemi *et al*. 2008). Thus, further studies may be

justified which deal with the eventual presence of FBV-1 on other host plants, and FBV-1 transmission by potential vectors in Iran. More genome sequences and greater numbers of isolates of FBV-1 from different hosts and geographical regions have to be included in future studies before definite conclusions can be made.

Understanding the genetic variation of FBV-1 populations, selective forces affecting the structure of viral populations, and epidemiology may well contribute to the design of improved disease management strategies and specific diagnostic tools.

Acknowledgements

We are grateful to Dr. Toufic Elbeaino [at: Istituto Agronomico Mediterraneo di Bari, Via Ceglie 9, 70010 Valenzano (BA), Italy] for providing valuable suggestions which added to this manuscript. The Islamic Azad University Scholarships Foundation is gratefully acknowledged for financial support of the presented work.

References

Acosta-Leal R., Duffy S., Xiong Z., Hammond R., Elena S.F. 2011. Advances in plant virus evolution: translating evolutionary insights into better disease management. Phytopathology 101 (10): 1136–1148.

Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z., Miller W., Lipman D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25 (17): 3389–3402.

Bouhida M., Lockhart B.E.L., Olszewski N.E. 1993. An analysis of the complete sequence of a sugarcane bacilliform virus genome infectious to banana and rice. Journal of General Virology 74 (1): 15–22.

Chabannes M., Iskra-Caruana M.L. 2013. Endogenous pararetroviruses a reservoir of virus infection in plants. Current Opinion in Virology 3 (6): 615–620.

Condit I.J., Horne W.T. 1933. A mosaic of the fig in California. Phytopathology 23 (11): 887–896.

Danesh-Amuz S., Rakhshandehroo F., Rezaee S. 2014. Prevalence and genetic diversity of fig mosaic virus isolates infecting fig tree in Iran. Acta Virologica 58 (3): 245–252.

Dellaporta S.L., Wood J.Y., Hicks J.B. 1983. A plant DNA minipreparation: version II. Plant Molecular Biology Reporter 1 (4): 19–21.

Dallot S., Acuna P., Rivera C., Ramirez P., Cote F., Lockhart B.E.L., Caruana M.L. 2001. Evidence that the proliferation stage of micropropagation procedure is determinant in the expression of Banana streak virus integrated into the genome of the FHIA 21 hybrid (Musa AAAB). Archive of Virology 146 (11): 2179–2190.

Elbeaino T., Digiaroa M., Martelli G.P. 2011a. Complete sequence of Fig fleck-associated virus, a novel member of the family Tymoviridae. Virus Research 161 (2): 198–202.

Elbeaino T., Kubaa R.A., Digiaro M., Minafra A., Martelli G.P. 2011b. The complete nucleotide sequence and genome organization of Fig cryptic virus, a novel bipartite dsRNA virus infecting fig, widely distributed in the Mediterranean basin. Virus Genes 42 (3): 415–421.

Elbeaino T., Gonzalez-Rodríguez A.M., Grajal-Martín M.J., Digiaro M. 2011c. Survey of fig viruses in the Canary Islands. Journal of Plant Pathology 93 (3): 737–739.

Elbeaino T., Abou Kubaa R., Ismaeil F., Mando J., Digiaro M. 2012. Viruses and *hop stunt viroid* of fig trees in Syria. Journal of Plant Pathology 94 (3): 687–691.

Elbeshehy E.K.F., Elbeaino T. 2011. Viruses infecting figs in Egypt. Phytopathologia Mediterranea 50 (2): 327–332.

Elci E., Ulubas Srce C., Gazel M., Caglayan K. 2012. Molecular detection and comparative sequence analysis of viruses infecting fig trees in Turkey. Journal of Phytopathology 160: 418–423.

Foissac X., Svanella-Dumas L., Dulucq M.J., Candresse T., Gentit P. 2001. Polyvalent detection of fruit tree tricho, capillo and foveavirus by nested RT-PCR using degenerated and inosine containing primers (DOP RT-PCR). Acta Horticulturae 550: 37–44.

Flock R., Wallace J. 1955. Transmission of fig mosaic by the eriophyid mite *Aceria ficus*. Phytopathology 45 (1): 52–54.

Gayral P., Noa-Carrazana J.C., Lescot M., Lheureux F., Lockhart B.E.L., Matsumoto T., Piffanelli P., Iskra-Caruana M.L. 2008. A single banana streak virus integration event in the banana genome as the origin of infectious endogenous pararetrovirus. Journal of Virology 82 (13): 6697–6710.

Grenfell B.T., Pybus O.G., Gog J.R., Wood J.L.N., Daly J.M., Mumford J.A., Holmes E.C. 2004. Unifying the epidemiological and evolutionary dynamics of pathogens. Science 303 (5656): 327–332.

Hearon S.S., Locke J.C. 1984. Graft, pollen, and seed transmission of an agent associated with top spotting in *Kalancöe blossfeldiana*. Phytopathology 74 (4): 347–350.

Iskra-Caruana M.L., Chabannes M., Duroy P.O., Muller E. 2014. A possible scenario for the evolution of *Banana streak virus* in banana. Virus Research 86: 155–162.

Laney A.G., Hassan M., Tzanetakis I.E. 2012. An integrated badnavirus is prevalent in fig germplasm. Phytopathology 102 (12): 1182–1189.

Liu R., Koyanagi K.O., Chen S., Kishima Y. 2012. Evolutionary force of AT-richrepeats to trap genomic and episomal DNAs into the rice genome: lessons fromendogenous pararetrovirus. Plant Journal 72 (5): 817–828.

Martelli G.P. 2011. Fig mosaic disease and associated pathogens. p. 281–287. In: "Virus and Virus-like Diseases of Pome and Stone Fruits" (A. Hadidi, M. Barba, T. Candresse, W. Jelkmann, eds.). APS Press, St. Paul, MN, USA, 428 pp.

Martin E.M., Kim K.S. 1987. A new type of virus causing striped chlorosis of mimosa. Phytopathology 77 (7): 935–940.

Medberry S.L., Lockhart B.E.L., Olszewski N.E. 1990. Properties of Commelina yellow mottle virus complete DNA sequence, genomic discontinuities and transcript suggest that it is a pararetrovirus. Nucleic Acids Research 18 (24): 5505–5513.

Meyer J.B., Kasdorf G.G.F., Nei L.H., Pietersen G. 2008. Transmission of activated-episomal *Banana streak OL* (*badna*) *virus* (BSOLV) to cv. Williams banana (*Musa* sp.) by three mealybugs species. Plant Disease 92 (8): 1158–1163.

Minafra A., Chiumenti M., Elbeaino T., Digiaro M., Bottalico G., Pantaleo V., Martelli G.P. 2012. Occurrence of Fig badnavirus 1 in fig trees from different countries and symptomless seedlings. Journal of Plant Pathology 94 (S4): 105.

Nei M., Kumar S. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York, 339 pp.

Norozian E., Rakhshandehroo F., Shams-bakhsh M. 2014. Presence of Fig leaf mottle associated virus 3 in an Iranian fig orchard. Journal of Plant Pathology 96 (S4): 113–131.

Nouri Ale-Agha G., Rakhshandehroo F. 2014. Detection and molecular variability of Fig fleck-associated virus and Fig cryptic virus in Iran. Journal of Phytopathology 162 (7–8): 417–425.

Oluwafemi S., Thottappilly G., Alegbejo M.D. 2008. Search for variability among masterviruses showing symptoms of Maize streak virus from different regions of Nigeria by polymerase chain reactions. Journal of Plant Protection Research 48 (4): 529–539.

Pamilo P., Bianchi N.O. 1993. Evolution of the Z fx and Z fy genes: rates and interdependence between the genes. Molecular Biology and Evolution 10 (2): 271–281.

Pearson W.R., Lipman D.J. 1988. Improved tools for biological sequence comparison. Proceedings of the National Academy of Sciences USA 85 (8): 2444–2448.

Saitou N., Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4 (4): 406–425.

Shahmirzaie M., Rakhshandehroo F., Zamanizadeh H.R., Elbeaino T. 2012. Current status of fig mosaic disease in Iran. Journal of Phytopathology 160 (8): 324–330.

Staginnus C., Iskra-Caruana M.L., Lockhart B., Hohn T., Richert-Poggeler K.R. 2009. Suggestions for a nomenclature of endogenous pararetroviral sequences in plants. Archive of Virology 154 (7): 1189–1193.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28 (10): 2731–2739.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123 (3): 585–595.

Walia J.J., Salem N.M., Falk B.W. 2009. Partial sequence and survey analysis identify a multipartite, negative-sense RNA virus associated with fig mosaic. Plant Disease 93 (1): 4–10.

Walia J.J., Willemsen A., Elci E., Caglayan K., Falk B.W., Rubio L. 2014. Genetic variation and possible mechanisms driving the evolution of worldwide *Fig mosaic virus* isolates. Phytopathology 104 (1): 108–114.