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SHORT COMMUNICATION

A SURVEY FOR FIG-INFECTING VIRUSES IN PALESTINE

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SUMMARY

A virus disease of fig (*Ficus carica*) known as fig mosaic (FM) is widely spread in Palestine, where its severity varies according to the cultivar and the growing area. At least 10 viruses and three viroids have been detected so far in fig trees. This study reports the results of a preliminary survey carried out in Palestine to secure information on the viruses associated with mosaic-infected figs. Samples were collected from scattered trees, fig orchards and nurseries of different areas of central Palestine, where figs are traditionally grown, and tested for the presence of *Fig mosaic virus* (FMV), *Fig latent virus 1* (FLV-1), *Fig badnavirus-1* (FBV-1), *Fig leaf mottle-associated virus 1* (FLMaV-1) and *2* (FLMaV-2), and Fig cryptic virus (FCV) in addition to *Apple dimple fruit viroid* (ADFVd). The following viruses. FMV, FBV-1, FLV-1 and FLMaV-2, were detected by RT-PCR. FBV-1 was the most widespread followed by FMV. The genetic diversity of FMV was assessed by sequencing a fragment from the viral p4 protein, revealing a low divergence from the homologous sequences from GenBank.

Key words: fig mosaic disease, fig-infecting viruses, molecular detection, diagnosis, field survey.

In Palestine, fig (*Ficus carica*), the same as olive, represents a symbolic plant for its cultural and religious importance. Figs are usually grown in areas where water is available. Scattered plants and specialized orchards coexist, the latter accounting for more than one thousand hectares. A mosaic condition of fig leaves characterized by varying patterns of chlorotic/yellowish discolourations has been described many decades ago, with which different viruses are associated (reviewed by Martelli, 2011). At least 10 viruses and three viroids have been detected and identified in mosaic-affected figs mainly in Italy (Table 1). These viruses include definitive or tentative species of the genera: *Closterovirus* [*Fig leaf mottle-associated virus 1* (FLMaV-1)], [*Fig mild mottle-associated virus* (FMMaV)], [Arkansas fig closterovirus 1 (AFCV-1)]; [Arkansas fig closterovirus 2 (AFCV-2)]; *Ampelovirus* [*Fig leaf mottle-associated virus 2* (FLMaV-2)]; *Trichovirus* [Fig latent virus 1 (FLV-1)]; *Alphacryptovirus* [Fig cryptic virus (FCV)]; *Maculavirus* [Fig fleck-associated virus (FFaV)]; *Emaravirus* [*Fig mosaic virus* (FMV)]; *Badnavirus* [*Fig badnavirus 1* (FBV-1)]. Fig-infecting viroids are: *Hop stunt viroid* (HSVd), *Citrus exocortis viroid* (CEVd) (Yakoubi *et al*., 2007) and *Apple dimple fruit viroid* (ADFVd) (Chiumenti *et al*., 2014).

Fig mosaic is common throughout Palestine, but no information is available on the viruses occurring in mosaic-affected plants. Thus, the present investigation represents the first attempt to look into the virus world of symptomatic Palestinian fig trees.

Table 1. Viruses and viroids identified in fig trees.

Infectious agents	Genus	Reference
Fig leaf mottle-associated virus 1 (FLMaV-1)	*Closterovirus*	Elbeaino *et al*., 2006
Fig leaf mottle-associated virus 2 (FLMaV-2)	*Ampelovirus*	Elbeaino *et al*., 2007
Fig mosaic virus (FMV)	*Emaravirus*	Elbeaino *et al*., 2009b
Fig latent virus 1 (FLV-1)	*Trichovirus*	Gattoni *et al*., 2009
Arkansas fig closterovirus-1 (AFCV-1)	*Closterovirus*	Tzanetakis and Martin, 2010
Arkansas fig closterovirus-2 (AFCV-2)	*Closterovirus*	Tzanetakis and Martin, 2010
Fig mild mottle-associated virus (FMMaV)	*Closterovirus*	Elbeaino *et al*., 2010
Fig cryptic virus (FCV)	*Alphacriptovirus*	Elbeaino *et al*., 2011b
Fig fleck-associated virus (FFkaV)	*Maculavirus*	Elbeaino *et al*., 2011a
Fig badnavirus-1 (FBV-1)	*Badnavirus*	Laney *et al*., 2012
Apple dimple fruit viroid (ADFVd)	*Apscaviroid*	Chiumenti *et al*., 2014
Citrus exocortis viroid (CEVd)	*Pospiviroid*	Yakoubi *et al*., 2007
Hop stunt viroid (HSVd)	*Hostuviroid*	Yakoubi *et al*., 2007

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Field surveys were carried out in Central Palestine (Jericho, Ramallah and Nablus) where figs are mostly grown, during late spring where the plant vegetation is at its peak. The type of field syndromes observed was in agreement with the descriptions from the world literature (Martelli, 2011) and varied among cultivars, ranging from moderate to extremely severe, including various patterns of leaf mottling and deformation, smaller and partially discolored fruits that dropped prematurely. The incidence of symptomatic trees in certain orchards reached 100% and was also very high in plants used for propagation by local nurseries, thus accounting for the widespread occurrence of mosaic in commercial stands.

Leaf petioles from symptomatic and symptomless trees were collected from 31 different trees and conserved at 4°C for later molecular investigations. Tissue samples (*ca.* 100 mg) were extracted according to Foissac *et al.* (2005) for recovering total nucleic acids (TNA) upon grinding in plastic bags/tubes containing 1 ml of buffer (4 M guanidine thiocyanate, 2 M sodium acetate, pH 5.2, 25 mM EDTA, 1 M potassium acetate, 2.5% PVP-40 and 2% sodium metabisulfite). Extracted TNA, after OD reading to estimate concentration, was analyzed by electrophoresis in 1.2% agarose gel in 1× TBE at 100 V (constant voltage). Gels were stained with GelRed (Biotium, USA) and observed with an UV-transilluminator

TNA aliquots (*ca.* 250 µg) were mixed with 150 ng of random hexamers (Roche, USA), denatured for 10 min at 70°C and kept on ice. Reverse transcription reaction was for 1 h at 42°C in a 20 µl vol of 50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 0.5 mM dNTPs, 200 units of reverse transcriptase (M-MLV, Life Technologies, USA). Two µl of the mixture were subjected to PCR amplification in 1× *Taq* polymerase buffer (Thermoscientific, USA), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 mM of both primers and 1 unit of Dreamtaq *Taq* DNA polymerase in a final volume of 25 µl. cDNAs were amplified in a thermal cycler apparatus (BioRad, USA) with 30 cycles at 94°C for 30 sec, 55°C for 45 sec, 72°C for 1 min. In the last cycle, extension at 72°C was for 7 min. Primers (Table 2) were selected based on the sequences available in the GenBank and published papers. Amplification products were analyzed in 1.2% agarose gel electrophoresis (Sambrook *et al.*, 1989). Gels were electrophoresed in Tris-acetate running buffer, pH 8.3, at 100 volt for 30 min. Amplified products were seen under UV light after staining with GelRed (Biotium, USA). Amplicons from 15 samples, testing positive for FMV, were selected for sequencing. PCR products were purified using the SAP and ExoI digestion (GE Healthcare, USA). Briefly, 2.5 µl of SAP/EXO master mix were added to each PCR reaction, the mixture was then incubated at 37°C for 45 min, followed by heating at 99°C for 15 min before cooling at 4°C. Then, 5 µl of the purified PCR product were custom-sequenced (Macrogen, The Netherlands), the obtained sequences were analysed by Clustal X (Thompson *et al.*, 1997) for multiple alignment and a phylogenetic tree was constructed using NJ-Plot program with a 1000 bootstrap replicates.

Successful amplifications were obtained using primers specific for FLMaV-2, FBV-1, FLV-1 and FMV but not for FLMaV-1, FMMaV and FCV-1 (Table 3). Fig latent virus-1 was detected only in two symptomless samples. The only viroid tested for (ADFVd) was not found.

FBV-1 was the most widespread virus detected in Palestinian figs, in accordance with its alleged worldwide incidence (Laney *et al.*, 2012; Minafra *et al.*, 2012), exemplified by its consistent identification in figs of many countries: i.e. Italy, New Zealand, France, Greece, Albania, Spain, Portugal, England, Hungary, Cuba, Australia, Lebanon, Syria, Tunisia, Algeria, and Turkey (Minafra *et al.*, 2012). The very high level of incidence (84%) in Palestine confirms the suggestion that the FBV-1 is integrated into the

Table 2. Fig viruses tested in this paper and PCR primers used to detect them.

Virus	Sequence		Amplicon	Reference
FMV	BB42up	5'- TGGCAGATTCAAGGATAATGG - 3'	218 bp	Elbeaino *et al.*, 2009b
	BB42down	5'- TGGGACATTCTTGTGTCAGG - 3'		
FLMaV-1	N17s	5'- CGTGGCTGATGCAAAGTTTA - 3'	350 bp	Elbeaino *et al.*, 2006
	N17a	5'- GTTAACGCATGCTTCCATGA -3'		
FLMaV-2	F3s	5'-GAACAGTGCCTATCAGTTTGATTTG-3'	360 bp	Elbeaino *et al.*, 2007a
	F3a	5'-TCCCACCTCCTGCGAAGCTAGAGAA-3'		
FBV-1	P1-s	5'-GCTGATCACAAGAGGCATGA-3	214 bp	Minafra *et al.*, 2012
	P1- as	5'-TCCTTGTTTCCACGTTCCTT-3'		
FLV-1	FFup	5'-CGCTTTGCCCCAATGTGCAGAT - 3'	194 bp	Gattoni *et al.*, 2009
	FFdown	5'- TCGAAAGCCAGTGTAGATGCATCC- 3'		
FCV	R1s	5'-TCGGATTGTCTTTGGAGAGG-3'	353 bp	Elbeaino *et al.*, 2011
	R1a	5'-CGCATCCACAGTATCCCATT-3'		
FMMaV	LM3s	5'-AAGGGGAATCTACAAGGGTCG-3'	311 bp	Elbeaino *et al.*, 2010
	LM3a	5'-TATTACGCGCTTGAGGATTGC-3'		
ADFVd	10-for	5'-CTCCGTGTGGTTCCTGTGGGGC-3'	274 bp	Chiumenti *et al.*, 2014
	ADFVd-rev	5'-GTGTTTTACCCTGGAGGCTCCACTC-3'		

fig genomes (Laney *et al.*, 2012). However, a reduced incidence of this virus was recorded in Palestinian fig plantlets obtained from newly propagated seedlings of different cultivars. For instance, only two cultivars (Khurtmani and Mwazi) out of six were found to host FBV-1. Whether this may be an indication that FBV-1 is differentially integrated in the genome of some cultivars but not in that of others remains to be investigated.

Fig mosaic disease (FMD) is graft- but not seed-transmissible (Condit and Horne, 1933; Blodgett and Gomec, 1967) and reported to have a variable symptomatology (Martelli *et al.*, 1993). As yet, no direct cause-effect relationship has been established between any of the recorded fig-infecting virused and FMD, the consensus is that FMV is one, if not the major disease agent (Elbeaino *et al.*, 2009a). This likelihood seems to be supported by the high incidence (61%) of FMV infections in symptomatic Palestinian trees, many of which showed signs of infestation by the FMV vector, the eriophyid mite (*Aceria ficus*) (see Martelli, 2011 and references therein). The FMV genome was found to be segmented and consist of four viral RNAs with a single open reading frame (7,093, 2,252, 1,490 and 1,472 nucleotide in size, respectively) for each. Primers on the virus RNA-4 portion were designed to amplify a 218 bp product as described by Elbeaino *et al.* (2009b), which was sequenced. The ensuing sequences showed up to 7% nucleotide variation among the Palestinian FMV isolates using the BLASTN 2.2 program (Altschul *et al.*, 1997). These variants occurred in samples collected from different regions as well as in some from the same areas (Fig. 1).

Viruses were usually found in symptomatic plants, except for a few cases in which samples from symptomless trees contained FBV-1 or FLV-1. However, in agreement with previous reports (Martelli, 2011), no clear-cut correlation could be established between field symptoms and specific viruses associated with them, since most of the samples hosted mixed infections.

Table 3. List of the Palestinian fig accessions tested, with their location or cultivar name.

Location\cultivars name	FMV	FBV	FLMaV-2	FLV-1
BeitWazan 1	–	–	+	–
BeitWazan 2	–	+	+	–
North Spring-Sarra 1	–	+	–	–
North Spring-Sarra 2	+	+	+	–
Sarra Spring-Nablus 1	–	+	+	–
Sarra Spring-Nablus 2	+	+	–	–
Sarra Spring-Nablus 3	+	+	–	–
Sarra Spring-Nablus 4	+	+	–	–
West Spring-Sarra 1	+	+	+	+
West Spring-Sarra 2	–	+	–	+
Fawwar Spring-Til 1	+	+	–	–
Fawwar Spring-Til 2	+	+	+	–
Fawwar Spring-Til 3	+	+	–	–
Fawwar Spring-Til 4	+	+	–	–
Fawwar Spring-Til 5	–	+	–	–
Till Valley 1	+	+	–	–
Till Valley 2	+	+	–	–
Till Valley 3	+	+	–	–
Till Valley 4	+	+	–	–
Till Valley 5	+	+	–	–
Local nursery-cultivar Swadi	–	–	–	–
Local nursery-cultivar Khurtmani	+	+	+	–
Local nursery-cultivar Mwazi	–	+	–	–
Local nursery-cultivar Qudsi	–	–	–	–
Local nursery-cultivar Einaki	–	–	–	–
Local nursery-cultivar Hmadi	–	–	–	–
Rafidia village 1	+	+	–	–
Rafidia village 2	+	+	–	–
Sabastia 1	+	+	–	–
Sabastia 2	–	+	–	–
Sabastia 3	+	+	–	–

Results of RT-PCR, observed by agarose gel electrophoresis of PCR products, are indicated by + (positive) or – (negative). For the viruses where only negative results were obtained, see the text.

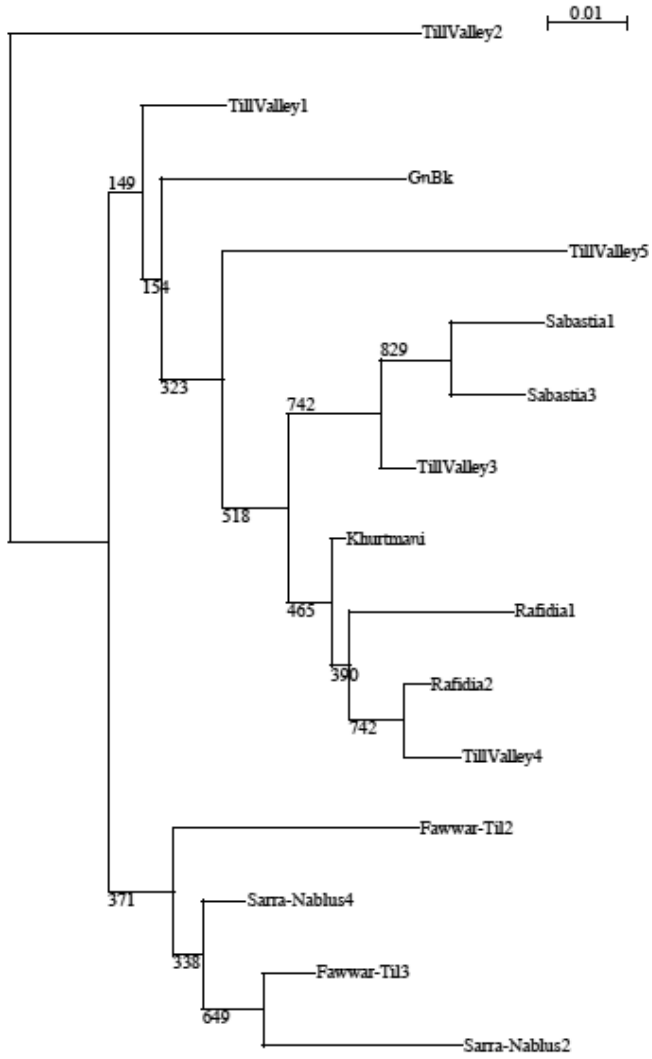

Fig. 1. Phylogenetic tree analysis showing nucleotide diversity and clustering inside the Palestinian FMV isolates in the amplified fragment of RNA4. A homologous fragment from a GenBank reference sequence (GnBk; accession N. FM992851) was included in the analysis. Bootstrap values are indicated at nodes. Bar represents nucleotide distance unit.

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