## SHORT COMMUNICATION

## PRESENCE OF FIG LEAF MOTTLE-ASSOCIATED VIRUS 1 AND 2 IN LEBANESE FIG ORCHARDS

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## **SUMMARY**

An account is given of the presence and incidence of Fig leaf mottle-associated virus 1 (FLMaV-1) and Fig leaf mottle-associated virus 2 (FLMaV-2) in Lebanese fig orchards, as determined by RT-PCR assays. About two-thirds of 102 fig trees tested were infected by at least one virus, with mixed infections in ca. 9% of the samples. FLMaV-1 prevailed (47% infection) especially in cv. Aswad (80% infection) and in Mount Lebanon (95% infection). FLMaV-2 was detected in 29.4% of the trees, with a prevalence in those coming from North Bekaa (57.5% infection). A fragment of the HSP70 gene of four FLMaV-1 and eight FLMaV-2 isolates were sequenced and aligned with the sequences of the homologous genes of the same viruses from Genbank. The comparative analysis showed low levels of variability among both FLMaV-1 (1-3%) and FLMaV-2 (3-9%). isolates.

Key words: Fig, fig mosaic, Closteroviridae, HSP70, RT-PCR.

Fig (*Ficus carica* L.) is widely grown in Lebanon on a surface of *ca.* 1900 ha (Anonymous, 2005) mainly as individual trees in gardens for family consumption, rarely in specialized orchards. A wide range of discolourations (chlorotic mottling and blotching, banding, clearing and feathering of the veins, chlorotic and necrotic ringspots and line patterns) and malformations of the leaves, resembling those typical of fig mosaic disease (FMD) (Appiano *et al.*, 1990), are commonly observed in fig trees throughout the country.

FMD is a graft-transmissible disease (Condit and Horne, 1933), thought to have a viral aetiology and to be vectored by the eriophyid mite *Aceria ficus* (Flock and Wallace, 1955; Slykhuis, 1973). Recently, two members of the family *Closteroviridae* were found in symptomatic figs of Italian and Algerian origin (Elbeaino *et al.*,

2006; 2007), which were denoted Fig leaf mottle-associated virus 1 (FLMaV-1) and Fig leaf mottle-associated virus 2 (FLMaV-2), respectively. Surveys carried out in Apulia (Southern Italy) and Tunisia, showed that both viruses were widespread. In particular, FLMaV-1 was detected in 64.9% of tested samples from Apulia (southern Italy) and 28.8% of samples from Tunisia (Elbeaino *et al.*, 2006; Nahdi *et al.*, 2006), whereas FLMaV-2 had an incidence of 13.5% in Tunisia (unpublished information).

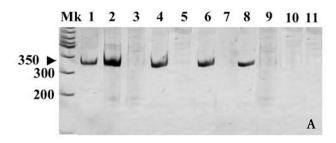
These findings prompted investigations on FMD-affected trees in the main fig growing areas of Lebanon, to look for the presence and incidence of the above closteroviruses. A survey was carried out in autumn 2006, collecting samples according to the importance and distribution of the cultivars in different areas of the country. Each sample consisted of 3-4 cuttings, about 30 cm in length, collected from one- to two-year-old vegetating twigs from the quadrant of the tree canopy. Samples were labelled and stored in plastic bags at 4°C until used for laboratory assays.

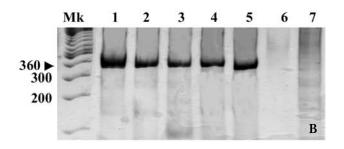
A total of 102 fig samples were collected, most of which (82) from the Bekaa, 47 in the north (El Saaidi, Boudai, Flaoui, Shlifa, Iaat, Baalbeck), 27 in the west (Jeb Jennine, Lala) and 8 in the centre (Zahle, Tal Amara) of the valley. The remaining 20 samples were from Mount Lebanon (Baskinta).

About 60% of the samples were from cv. Biadi, which is the most important fig variety in the country, whereas the rest of the samples were from cv. Aswad (24.5%), Houmairi (8.8%), and some unknown varieties (6.9%).

Virus detection was by RT-PCR using the virus-specific primers designed on the HSP70 gene sequence of FLMaV-1 (N17s: 5'-CGTGGCTGATGCAAAGTTTA-3'; N17-a: 5'-GTTAACGCATGCTTCCATGA-3') and FLMaV-2 (F3-s: 5'-GAACAGTGCCTATCAGTTTGATTTG-3'; F3-a: 5'-TCCCACCTCCTGCGAAGCTAGA-GAA-3'), which amplified DNA fragments of 350 bp and 360 bp for FLMaV-1 and FLMaV-2, respectively (Elbeaino *et al.*, 2006, 2007).

Total nucleic acids (TNAs) were extracted from 100 mg tissue from leaf veins or cortical scrapings macerated in 1 ml grinding buffer (4.0 M guanidine thio-





**Fig. 1.** RT-PCR products amplified from total nucleic acid extracts from leaf tissues of field-grown symptomatic fig trees. **A.** FLMaV-1 positive samples (lanes 2, 4, 6 and 8). Negative samples (lanes 3, 5, 7, 10 and 11). Controls: positive (lane 1), water (lane 9). **B.** FLMaV-2 positive samples (lanes 2, 3, 4 and 5). Negative sample (lane 6). Controls: positive (lane 1), water (lane 7). Mk = markers.

cyanate, 0.2 M NaOAc pH 5.2, 25 mM EDTA, 1.0 M KOAc and 2.5% w/v PVP-40), recovered with a silicacapture procedure (Foissac *et al.*, 2001), and stored at –20°C until used.

Eight to 10μl of TNA extract were mixed with 1μl random hexamer primer, (Boehringer Mannheim, Germany) (0.5 μg/μl), denatured at 95°C for 5 min and quickly chilled in ice. Reverse transcription reaction was done for 1h at 39°C by adding 4μl M-MLV buffer 5x (50 mM tris-HCl pH 8.3, 75 mM KCl, 3 mM MgCl<sub>2</sub>), 2μl of 10 mM DTT, 0.5μl of 10 mM dNTPs, and 200 units Moloney Murine Leukaemia virus (M-MLV) reverse transcriptase (Bethesda Research Laboratories, USA) in a final volume of 20 μl.

For RT-PCR, 2.5  $\mu$ l cDNA mixture were submitted to amplification by adding 2.5  $\mu$ l of 10xTaq polymerase buffer (Promega, Madison WI, USA), 1.5 mM as final concentration of MgCl<sub>2</sub>, 0.5  $\mu$ l of 10 mM dNTPs, 0.5 $\mu$ l of 10  $\mu$ M sense and antisense of each specific primers, and 0.2  $\mu$ l of Taq polymerase (5 unit/ $\mu$ l) in a final volume of 25  $\mu$ l.

Amplicons were cleaned by Nucleospin (Macherey-Nagel, Germany) and cloned into pGEM-T Easy vector (Promega, Madison WI, USA), according to the manufacturers' instructions. The nucleotide sequence of PCR amplicons of four different FLMaV-1 isolates and eight FLMaV-2 isolates was determined, to assess the variability level. Sequences were compared with those of the homologous genes of FLMaV-1 (Acc. No. AM113547) and FLMaV-2 (Acc. No. AM286422) from Genbank.

Nucleotide and amino acid sequences were assembled with Strider 1.1 program (Marck, 1988) and aligned with Clustal X program. Homology with known HSP70 amino acid sequences of FLMaV-1 and FLMaV-2 was determined using FASTA (Pearson and Lipman, 1988) and BLASTA (Altschul *et al.*, 1990) programs.

According to RT-PCR results (Fig. 1), ca. 68% of the tested plants were infected by at least one virus. FLMaV-1 occurred in all the surveyed regions with infection rates ranging from 12.5% (Central Bekaa) to 95% (Mount Lebanon) (Table 1). Infections were particularly high in cv. Aswad (80%) and Houmairi (55.6%), but less significant in cv Biadi (34.4%) and in the undetermined varieties (28.6%) (Table 2).

FLMaV-2 was less represented, since it was detected only in 29.4% of the tested samples (Table 1). This virus was widespread in north Bekaa (57.5% infections), less common in Mount Lebanon (15% infection) and completely absent in the other areas surveyed (Table 1). Biadi, the most important variety in north Bekaa, was heavily infected with FLMaV-2 (39.3%), but infection rates were lower in cvs Aswad (20%) and Houmairi (11.1%) (Table 2).

About 9% of the surveyed trees were infected by both viruses, particularly in north Bekaa and Mount Lebanon (14.9% and 10.0%, respectively), with a prevalence in cvs Aswad (16.0%) and Biadi (8.2%).

The alignment of nucleotide sequences (not shown) of twelve FLMaV-1 and FLMaV-2 Lebanese isolates with the corresponding HSP70 gene sequence from

Table 1. Infections by FLMaV-1 and FLMaV-2 detected by RT-PCR in different Lebanese areas.

D 1	Tested trees	Infected trees		FLMaV-1		FLMaV-2	
Regions	No.	No.	%	No.	%	No.	%
Mount Lebanon	20	20	100	19 ª	95	3 a	15
North Bekaa	47	37	78,7	17 <sup>ь</sup>	36.2	27 <sup>b</sup>	57.5
West Bekaa	27	11	40,7	11	40,7	0	0
Central Bekaa	8	1	12,5	1	12,5	0	0
Total	102	69		48 °		30 °	
Mean infection rate			67,6		47.0		29.4

<sup>&</sup>lt;sup>a</sup> 2 mixed infected samples

<sup>&</sup>lt;sup>b</sup> 7 mixed infected samples

<sup>&</sup>lt;sup>c</sup> 9 mixed infected samples

Table 2. Infections by FLMaV-1 and FLMaV-2 detected by RT-PCR in different Lebanese cultivars.

T	Tested trees	Infected trees		FLMaV-1		FLMaV-2	
Varieties	No.	No.	%	No.	%	No.	%
Biadi	61	40	65,6	21 a	34.4	24 a	39.3
Aswad	25	21	84	20 b	80	5 <sup>ь</sup>	20
Houmairi	9	6	66,7	5	55,6	1	11,1
Unknown	7	2	28,6	2	28,6	0	0
Total	102	69		48 <sup>c</sup>		30 °	
Mean infection rate			67.6		47.0		29.4

<sup>&</sup>lt;sup>a</sup> 5 mixed infected samples

Genbank showed nucleotide variations from 1% to 2% for FLMaV-1 and from 4% to 8% for FLMaV-2. The intra-variability among these isolates ranged from 1 to 3% for FLMaV-1 and from 3 to 9% for FLMaV-2.

The positive detection of FLMaV-1 and FLMaV-2 in symptomatic fig trees from Lebanon confirms the association of both viruses with FMD, already reported from Italy (Elbeaino et al., 2006, 2007) and other countries (Falk and Salem, 2006; Nahdi et al., 2006) and calls for further studies aimed at establishing the role, if any, of these viruses in the aetiology of FMD.

## REFERENCES

Altschul S.F., Stephen F., Gish W., Miller W., Myers E.W., Lipman D.J., 1990. Basic local alignment search tool. Journal of Molecular Biology **215**: 403-410.

Anonymous, 2005. L'agriculture au Liban 2004. Projet de recensement agricole. Projet FAO. 126 p.

Appiano A., Conti M., Lovisolo O., 1990. Mosaico del fico: stato attuale delle conoscenze e nuove osservazioni. Agricoltura Ricerca 112-113: 109-112.

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

Elbeaino T., Digiaro M., De Stradis A., Martelli G.P., 2006. Partial characterization of a closterovirus associated with a chlorotic mottling of fig. Journal of Plant Pathology 88: 187-192.

Elbeaino T., Digiaro M., De Stradis A., Martelli G.P., 2007. Identification of a second member of the family Closteroviridae in mosaic-diseased figs. Journal of Plant Pathology 89: 119-124.

Falk B.W., Salem N., 2006. Fig mosaic. http://fruitsandnuts.ucdavis.edu/crops/2006figday\_falk.pdf.

Flock R.A., Wallace J.M., 1955. Transmission of fig mosaic by the eriophid mite Aceria ficus. Phytopathology 45: 52-54.

Foissac X., Svanella-Dumas L., Gentit P., Dulucq M.J., Candresse T., 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 (ISHS) 550: 37-43.

Marck C. 1988. "DNA Strider": a "C" programme for the fast analysis of DNA and protein sequences on the Apple Macintosh family computers. Nucleic Acids Research 16: 1829-

Nahdi S., Elbeaino T., Digiaro M., Martelli G.P., 2006. First record of Fig leaf mottle-associated virus 1 in Tunisia. Journal of Plant Pathology 88 (3, supplement): S70.

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

Slykhuis J.T., 1973. Viruses and mites. In: Gibbs A.J. (ed.). Viruses and Invertebrates, pp. 391-405. North Holland Publishing Co., Amsterdam, The Netherlands.

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<sup>&</sup>lt;sup>b</sup> 4 mixed infected samples

<sup>&</sup>lt;sup>c</sup> 9 mixed infected samples