Survey on Fig Viruses in Lebanon

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Keywords: *Ficus carica*, Lebanon, virus survey, RT-PCR

Abstract

A survey was carried out in the main fig-growing areas of Lebanon (Bekaa and Mount Lebanon) in 2006-2008. A total of 102 samples were collected and tested by RT-PCR for the presence of *Fig mosaic virus* (FMV), *Fig leaf mottle-associated virus 1* (FLMaV-1), *Fig leaf mottle-associated virus 2* (FLMaV-2), *Fig mild mottle associated virus* (FMMaV) and a still unclassified isometric virus of the *Tymoviridae* family (hereafter indicated as FFkaV) using virus-specific primers. About 90% of the trees were infected with at least one virus, with mixed infections in ca. 46% of the samples. FLMaV-1 *closterovirus* was the prevailing virus (47% infection), especially in Mount Lebanon (95%) and on 'Aswad' (80%), followed by FMV *Emaravirus* (42.2% infection), which was particularly widespread in North Bekaa (68.1%) on 'Biadi' (50.8%). Two more viruses of the family *Closteroviridae*, FLMaV-2 and FMMaV, were detected respectively in 29.4 and 26.5% of the samples, with FLMaV-2 particularly widespread in north Bekaa (57.4%) on 'Biadi' (39.4%). FFkaV was detected in 13.7% of the samples, with an infection peak in 'Houmairi' (40%).

INTRODUCTION

Fig (*Ficus carica* L.) is widely cultivated throughout the Lebanese territory, mainly as individual trees in gardens and orchards for familiar consumption, and only rarely as specialized crops. According to statistics, the fig trees in Lebanon cover a surface area of ca. 1862 ha, for a total estimated production of 9.6×10^3 T (Anonymous, 2005). Symptoms observation in Lebanese fig orchards showed the presence of a wide range of foliar discolouration (chlorotic mottling and blotching, banding, clearing and feathering of the veins, chlorotic and necrotic ringspots and line patterns) and malformation, resembling those typical of fig mosaic disease (FMD) (Appiano et al., 1995). These symptoms were commonly observed on fig trees throughout the country (Fig. 1). Accordingly, an investigation on the presence of fig viral diseases was conducted in the country. The viruses investigated were the following: *Fig leaf mottle-associated virus 1* (FLMaV-1), *Fig leaf mottle-associated virus 2* (FLMaV-2), *Fig mild mottle-associated virus* (FMMaV), *Fig mosaic virus* (FMV) and an isometric still unclassified virus of the *Tymoviridae* family, hereafter indicated as *Fig fleck associated virus* (FFkaV) (Elbeaino et al., 2006, 2007a, 2009, 2010).

MATERIALS AND METHODS

Virus Sources

A total of 102 fig samples were collected during a preliminary field survey in the main fig-growing areas of Lebanon (Bekaa and Mount Lebanon) in 2006-2008 (Elbeaino et al., 2007b). Most of the samples (82) were collected from the Beckaa valley, 47 of which at north (El Saaidi, Boudai, Flaoui, Shlifa, Iaat, Baalbeck), 27 at west (Jeb Jennine, Lala) and 8 at centre (Zahle, Tal Amara). The remaining 20 samples were collected from Mount Lebanon region (Baskinta).

About 60% of the collected samples were from 'Biadi', considered as the most widespread and representative fig cultivar in the country. All the remaining samples were from 'Aswad' (24.5%), 'Houmairi' (9.8%), and some other unknown cultivars (5.9%).

Proc. XXVIIIth IHC – IS on the Challenge for a Sustainable Production,
Protection and Consumption of Mediterranean Fruits and Nuts
Eds.: A.M. D'Onghia et al.
Acta Hort. 940, ISHS 2012

TNA Extraction, cDNA Synthesis and PCR

Total nucleic acids (TNAs) were extracted from leaf veins or cortical scrapings of fig samples. One hundred mg of tissue were macerated in 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 TNAs were recovered with a silica-capture procedure as described by Foissac et al. (2001) and stored at -20°C until used.

From 8 to 10 µl of TNA extract were mixed with 1 µl random hexamer primer (Boehringer Mannheim, GbmH, Germany) (0.5 µg/µl), denatured at 95°C for 5 min and quickly chilled in ice. Reverse transcription reaction was done for 1 h at 39°C by adding 4 µl M-MLV buffer 5× (50 mM tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$), 2 µl of 10 mM DTT, 0.5 µl of 10 mM dNTPs, and 200 units Moloney Murine Leukaemia virus (M-MLV) reverse transcriptase (Invitrogen Corp., Milan, Italy) in a final volume of 20 µl. 2.5 µl of the cDNA mixture were submitted to PCR amplification by adding 2.5 µl of 10× Taq polymerase buffer (Promega Corp., Madison, USA), 1.5 mM as final concentration of $MgCl_2$, 0.5 µl of 10 mM dNTPs, 0.5 µl of 10 µM sense and antisense of each specific primers (Table 1), and 0.2 µl of Taq polymerase (5 unit/µl) in final volume of 25 µl.

RESULTS AND DISCUSSION

About 90% of the trees were infected by at least one virus, with mixed infections in ca. 46% of the samples. FLMaV-1 *closterovirus* was the prevailing virus (47% infection), especially in Mount Lebanon (95%) and on 'Aswad' (80%), followed by FMV *Emaravirus* (42.2% infection), which was particularly spread in North Bekaa (68.1%) on 'Biadi' (50.8%). Other two viruses of the family *Closteroviridae*, FLMaV-2 and FMMaV, were detected respectively in 29.4 and 26.5% of the samples, with FLMaV-2 particularly spread in north Bekaa (57.4%) on 'Biadi' (39.3%). FFkaV was detected in 13.7% of samples, with peak of infection of 40% in 'Houmairi'.

The results of this preliminary survey conducted in Lebanon have shown a very deteriorated sanitary status of the fig crop (over 90% of viral infections). This is not surprising considering the mode of propagation of this species (by cuttings and grafting) and the presence of very efficient virus vectors (eriophyid mites, mealy bugs and aphids). The knowledge gained in recent years on virus diseases of fig can finally allow to initiate the sanitary selection, sanitation and certification of plant propagating materials programs, which can count on the support of molecular specific diagnostic tools (PCR).

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Tables

Table 1. List of primers used in RT-PCR for the detection of fig-infecting viruses.

Viruses	Primers sequenze	Amplicon size (bp)
FLMaV-1	5' CGTGGCTGATGCAAAGTTT 3' 5'GTTAACGCATGCTTCCATGA 3'	352
FLMaV-2	5'GAACAGTGCCTATCAGTTTGATTTG 3' 5'TCCCACCTCCTGCGAAGCTAGAGAA 3'	360
FMMaV	5'AAGGGGAATCTACAAGGGTCG 3' 5'TATTACGCGCTTGAGGATTGC 3'	311
FMV	5'CGGTAGCAAATGGAATGAAA 3' 5'AACACTGTTTTTGCGATTGG 3'	302

Table 2. Distribution of fig-infecting viruses in different Lebanese areas.

Regions	Tested trees (no.)	Infected trees (%)	FLMaV-1		FLMaV-2		FMMaV		FMV		FFkV	
			No	%	No	%	No	%	No	%	No	%
Mount Lebanon	20	100	19	95	3	15	1	5	5	25	2	10
Central Bekaa	8	78.7	1	12.5	0	0	3	37.5	2	25	1	12.5
West Bekaa	27	81.5	11	40.7	0	0	10	37	4	14.8	6	22.2
North Bekaa	47	93.6	16	34	27	57.4	13	27.7	32	68.1	5	10.6
Total	102	90.2	48	47.0	30	29.4	27	26.5	43	42.2	14	13.7

Table 3. Incidence of fig-infecting viruses in Lebanese fig cultivars.

Cultivars	Tested trees (no.)	Infected trees (%)	FLMaV-1		FLMaV-2		FMMaV		FMV		FFkV	
			No	%	No	%	No	%	No	%	No	%
Aswad	25	96	20	80	5	20	3	12	9	36	2	8
Biadi	61	90.2	20	32.8	24	39.3	21	34.4	31	50.8	8	13.1
Houmairi	10	90	6	60	1	10	1	10	2	20	4	40
Unknown	6	33.3	2	33.3	0	0	2	33.3	1	16.7	0	0
Total	102	90.2	48	47.1	30	29.4	27	26.5	43	42.2	14	13.7

Figures

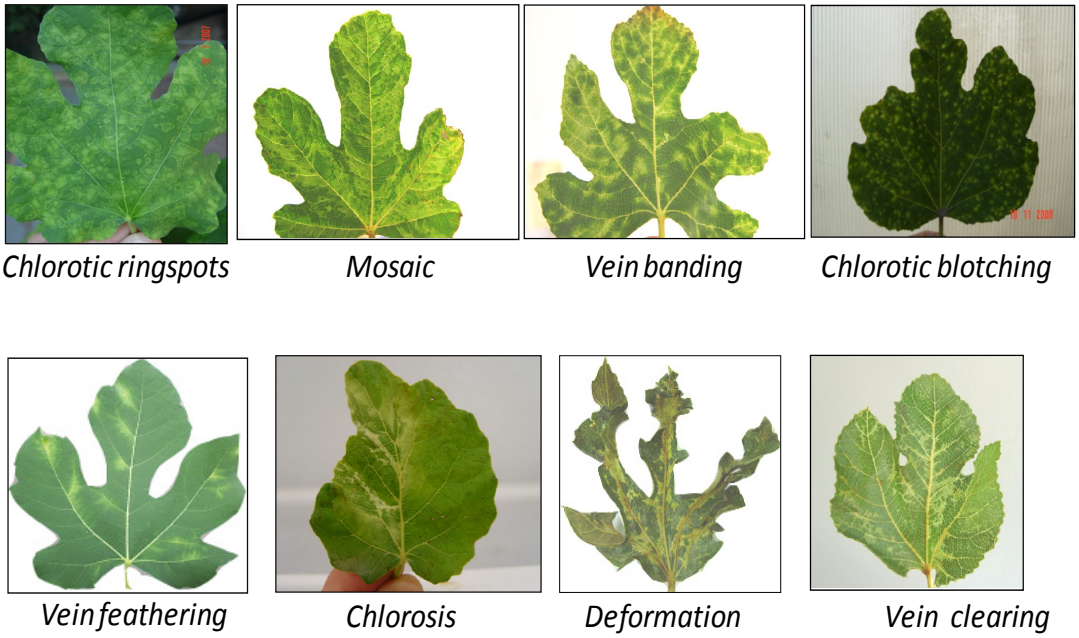

Fig. 1. Symptoms observed in the surveyed fig orchards.

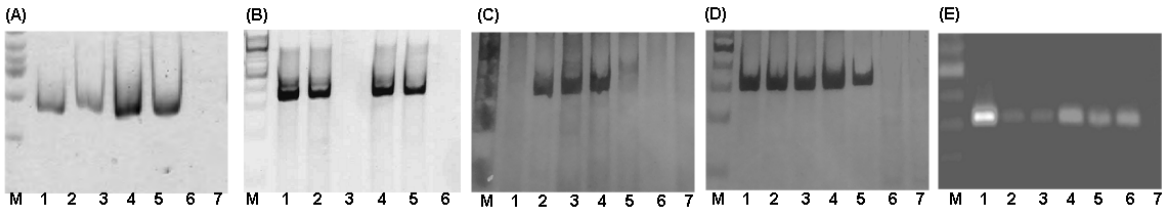

Fig. 2. Electropherogram of polyacrylamide gel showing PCR amplifications of some fig infected samples. (A) FLMaV-1, (B) FLMaV-2, (C) FMMaV, (D) FMV and (E) FFkaV.