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samples from Tunisia (Elbeaino et al., 2006; Nahdi et al., 2006), whereas FLMaV-2 had an incidence of 13.5% in Tunisia (unpublished information).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’-CGTGGCCTGATGCAAAGTTTA-3’; N17-a: 5’-GTTAACGCATGCTTCCATGA-3’), and FLMaV-2 (F3-s: 5’-GAACAGTGCCTATCAGTTTGA-3’; F3-a: 5’-TCCCACCTCCTGCGAAGCTAGA-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-
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 silica-capture 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-ML V buffer 5x (50 mM tris-HCl pH 8.3, 75 mM KCl, 3 mM MgCl2), 2µl of 10 mM DTT, 0.5µl of 10 mM dNTPs, and 200 units Moloney Murine Leukaemia virus (M-ML V) reverse transcriptase (Bethesda Research Laboratories, USA) in a final volume of 20 µl.

For RT-PCR, 2.5 µl cDNA mixture were submitted to amplification by adding 2.5 µl of 10xTaq polymerase buffer (Promega, Madison WI, USA), 1.5 mM as final concentration of MgCl2, 0.5 µl of 10 mM dNTPs, 0.5µl of 10 µM sense and antisense of each specific primers, and 0.2 µl of Taq polymerase (5 unit/µl) in a final volume of 25 µl.

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

![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.](image-url)
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.

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

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Tested trees No.</th>
<th>Infected trees No.</th>
<th>FLMaV-1 %</th>
<th>FLMaV-2 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biadi</td>
<td>61</td>
<td>40</td>
<td>65.6</td>
<td>21*</td>
</tr>
<tr>
<td>Aswad</td>
<td>25</td>
<td>21</td>
<td>84</td>
<td>20b</td>
</tr>
<tr>
<td>Houmairi</td>
<td>9</td>
<td>6</td>
<td>66.7</td>
<td>5</td>
</tr>
<tr>
<td>Unknown</td>
<td>7</td>
<td>2</td>
<td>28.6</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>102</td>
<td>69</td>
<td>48c</td>
<td>30c</td>
</tr>
</tbody>
</table>

* 5 mixed infected samples  
* 4 mixed infected samples  
* 9 mixed infected samples

Received May 10, 2007  
Accepted June 20, 2007

REFERENCES


