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# Viruses infecting figs in Egypt

Esam Kamal Fahmy ELBESHEHY [1] and Toufic ELBEAINO [2]

[1]Agricultural Botany Department, Faculty of Agriculture, Suez Canal University, 41522 Ismailia, Egypt
[2] Istituto Agronomico Mediterraneo di Bari, Via Ceglie 9, 70010 Valenzano (Bari), Italy

**Summary.** Fig production in Egypt is in continuous decline because many diseases, particularly those caused by viruses, are compromising this crop. RT-PCR assays were conducted on 60 fig samples collected from three Egyptian fig-growing provinces (Ismailia, Qena and North Sinai), from the three main fig varieties (cvs. Sultany, Abode and El-Adasy), to investigate the presence of *Fig leaf mottle-associated virus 1* (FLMaV-1), *Fig leaf mottle-associated virus 2* (FLMaV-2), *Fig mild mottle-associated virus* (FMMaV) and *Fig mosaic virus* (FMV). The overall average incidence of infection reached 90%, with a peak of 95% in Ismailia province. All four viruses were detected in tested samples, with infection rates of 68.3% for FLMaV-1, 35% for FLMaV-2, 28.3% for FMMaV and 46.7% for FMV. This is the first report of the presence of these viruses in Egypt and offers a preliminary insight on the unsatisfactory sanitary status of fig in this country.

**Key words**: *Ficus carica*, FMV, virus detection, RT-PCR, electron microscopy.

## Introduction

According to FAO statistics, Egypt is the second largest fig producer in the world, after Turkey, with a production of 170,000 tons (Anonymous, 2005). More than 5 million fig trees are grown in Egypt on a total area of 32,060 ha (General Administration for Agriculture, Statistics, MOA, Egypt). In the last few years, poor growth of fig trees, scant yield and low fruit quality have been common complaints from growers in different regions of Egypt. The unknown sanitary status of fig crops in Egypt, together with the traditional techniques for fig propagation (grafting and/or self-rooted cuttings), have favored the spread of pathogens, especially viruses. Symptoms of mosaic disease, always presumed and only recently confirmed to be of viral origin (Elbeaino *et al*., 2009a), are scattered in gardens as well as in commercial fig orchards throughout the country. The first description of fig mosaic disease (MD) was from California by Condit and Horn (1933), and this disease was found to be transmitted by grafting and in nature by the eryophid mite *Aceria ficus* (Flock and Wallace, 1955). The aetiology of this disease remained uncertain for a long time, even though isometric and filamentous virus particles were occasionally observed in thin-sectioned tissues of symptomatic leaves of fig accessions from Italy and England (Martelli *et al*., 1993). Putative potyviruses were also recorded from Croatia (Grbelja and Eric, 1983) and Spain (Serrano *et al*., 2004), a putative carlavirus from Japan (Doi, 1989), and double stranded RNAs (dsRNA) with sizes ranging from 0.6 to 6.6 kb were recovered from infected trees in Portugal and Turkey (Nolasco and Sequeira, 1991; Açikgös and Döken, 2003).

A closterovirus denoted *Fig leaf mottle-associated virus 1* (FLMaV-1) was the first virus infecting fig in nature whose partial nucleotide sequence was determined and submitted in Genbank (Elbeaino *et al*., 2006). Later records of

Corresponding author: T. Elbeaino
Fax: +39 080 4606275
E-mail: elbeaino@iamb.it

fig-infecting viruses rapidly increased and additional viruses infecting fig joined the list: *Fig leaf mottle-associated virus 2* (FLMaV-2), *Fig mosaic virus* (FMV), *Fig latent virus 1* (FLV-1), *Fig mild mottle-associated virus* (FMMaV) and *Fig cryptic virus* (FCV) (Elbeaino *et al.,* 2007; 2009a; 2010; 2011; Gattoni *et al*., 2009). Contemporarily, other viruses likely belonging to *Partitiviridae* (Luteovirus-like) and *Caulimoviridae* (Badnavirus-like) families, with scanty molecular information (short nucleotide sequences) of their genomes, were also found in infected diseased figs (Walia *et al*., 2009, Tzanetakis *et al*., 2009). For all of these viruses, molecular information on genome organization and sequences which permit their detection using molecular tools are now available. In this paper we report a preliminary investigation carried out in some fig-growing areas of southern Egypt to assess the presence of FLMaV-1, FLMaV-2, FMMaV and FMV using molecular tools (RT-PCR).

## Materials and methods

### Field survey and plant material

A total of sixty samples was collected from naturally infected fig trees of cvs. Sultany, Abode and El-Adasy in the three fig-growing provinces of Egypt, Ismailia, Qena and North Sinai. Sampling was conducted during May 2010, and collections were made both from symptomless and MD-symptomatic trees. Symptoms varied from chlorotic blotches, vein clearing, vein banding, chlorosis, mosaic and chlorotic ringspot, all suggestive of virus-like diseases (Figure 1). All of the samples were assayed by RT-PCR to assess the presence of FLMaV-1, FLMaV-2, FMMaV and FMV in Egypt.

### Total nucleic acid extraction (TNA)

Total nucleic acids (TNAs) were extracted from 100 mg of leaf veins or cortical scrapings by ground each sample in 1 mL of grinding buffer (4.0M guanidine thiocyanate, 0.2M NaOAc pH 5.2, 25mM EDTA, 1.0M KOAc and 2.5% (w/v) PVP-40), and were silica-purified (Foissac *et al*., 2001).

### cDNA synthesis

Eight to 10 μL of TNA extracts were mixed with 1 μL of random hexamer primers, (Boehringer Mannheim GbmH, Mannheim, Germany) (0.5 μg $\mu L^{-1}$ ), denatured at 95°C for 5 min and quickly chilled on ice. Reverse-transcription 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 10mM DTT, 0.5 μL of 10 mM dNTPs, and

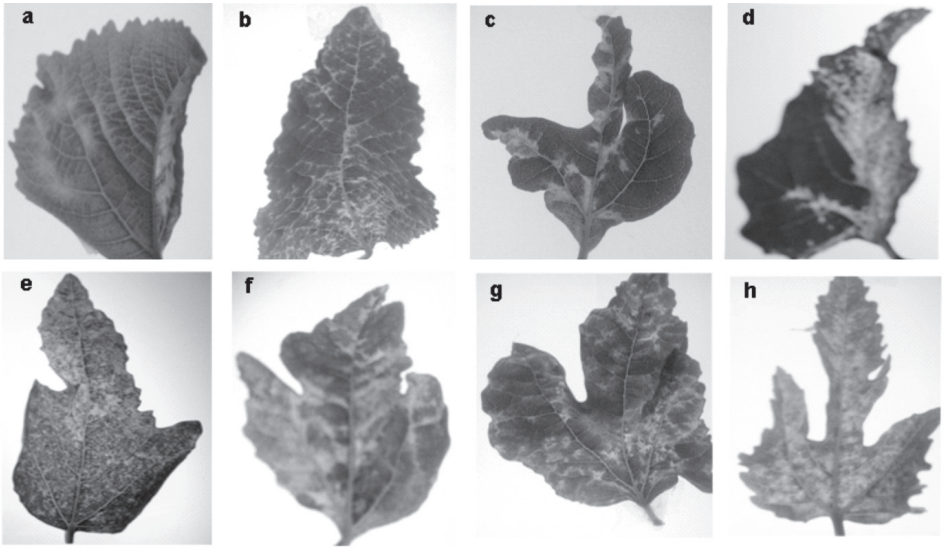


Figure 1. Symptoms of fig-mosaic diseased plants observed in inspected fig orchards and showing a wide range of foliar discoloration and malformation symptoms. (a) vein feathering, (b) vein banding, (c) leaf deformation, (d) chlorosis, (e) mosaic, (f) chlorotic blotching, (g) chlorotic ringspot and (h) leaf puckering.

200 units of *Moloney Murine Leukaemia virus* (M-MLV) reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, M, USA) in a final volume of 20 µL.

**PCR**

The detection of FLMaV-1, FLMaV-2, FMMaV and FMV in RT-PCR was conducted using four sets of specific primers, whose nucleotide sequences and use-conditions were previously described (Elbeaino *et al.,* 2006, 2007, 2009a, 2010). Briefly, for each sample, 2.5 µL of reverse-transcribed TNA mixture was submitted to amplification with the addition of 2.5 µL of 10× Taq polymerase buffer (Promega Corporation, Madison, WI, USA), with a final concentration of 1.5 mM $MgCl_2$ for a a total volume of 25 µL. PCR products were analyzed by electrophoresis on 1.2% agarose gel prepared in 1× TBE buffer (Sambrook *et al*., 1989), stained with ethidium bromide and examined on a UV transilluminator.

**Electron microscope**

For thin sectioning, tissue pieces from veins and mesophyll tissues of the discolored areas of young leaves were processed according to standard procedures (Martelli and Russo, 1984), i.e. fixation in 4% glutaraldehyde in 0.05M phosphate buffer for 2 h, post-fixation in 1% osmium tetroxide for 2 h, staining overnight in 2% aqueous uranyl acetate, dehydration in ethanol, and embedding in Spurr's medium. Thin sections were stained with lead citrate and viewed with a JOEL-JEA100 CX electron microscope Unit (Faculty of Science, Zagazig University, Alsharqiya, Egypt). Controls consisted of leaf tissues from a PCR-negative fig seedling processed as above.

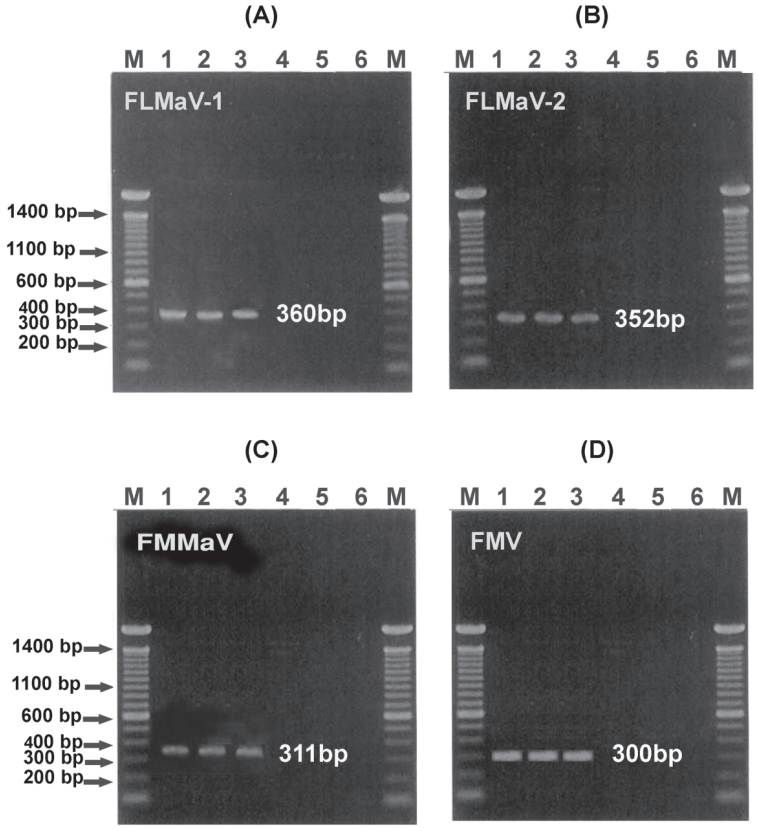


Figure 2. Electropherogram of polyacrylamide gel showing PCR amplifications from infected figs. (A) FLMaV-1, ( B) FLMaV-2, (C) FMMaV and (D) FMV. Lane M, DNA ladder marker, Lanes 1, 2 and 3 represent PCR-positive infected fig plants. Lanes 4 and 5 represent healthy figs.

Table 1. Incidence of *FLMaV-1*, *FLMaV-2*, *FMMaV* and FMV infections in three fig-growing provinces of Egypt as determined by RT-PCR assays.

| Province | Tested trees No. | Infected trees | | *FLMaV-1* | | *FLMaV-2* | | *FMMaV* | | FMV | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | No. | % | No. | % |
| Ismailia | 20 | 19 | 95 | 18 | 90 | 5 | 25 | 3 | 15 | 9 | 45 |
| Qena | 20 | 17 | 85 | 9 | 45 | 9 | 45 | 7 | 35 | 10 | 50 |
| Sinai | 20 | 18 | 90 | 14 | 70 | 7 | 35 | 7 | 35 | 9 | 45 |
| Total | 60 | 54 | 90 | 41 | | 21 | | 17 | | 28 | |
| Mean infection rate | | | 90 | | 68.3 | | 35 | | 28.3 | | 46.7 |

## Results and discussion

### RT-PCR Detection

RT-PCR assays of samples yielded four DNA amplifications of expected sizes, 352 bp, 360 bp, 311 bp and 300 bp, as results for FLMaV-1, FLMaV-2, FMMaV and FMV infections, respectively (Figure 2). From a total of 60 samples, 54 (90%) were infected by at least one virus. FLMaV-1 was the prevailing virus with an infection rate of 68.3% (Table 1). The incidence of this virus was particularly high on cv. Sultany grown in Ismailia province (90%) and on cv. El-Adasy in Sinai (70%). FMV ranked second as incidence (46.7%) and was substantially equally distributed in all cultivars and regions (45–50%). FLMaV-2 and FMMaV, the other two viruses checked in this study, although to a lesser extent were found in all three provinces, with peaks of infection of 45% for FLMaV-2 in Qena province and of 35% for FMMaV in Qena and Sinai provinces. These results are in harmony with those previously reported in other Mediterranean countries (Elbeaino *et al*., 2006; 2007; 2009b; 2009c). Unlike closterovirus infections, which were frequently detected in asymptomatic trees, all FMV PCR-positive samples were closely correlated with mosaic symptoms in diseased fig plants.

### Electron microscope

Electron micrographs of sectioned cells revealed the presence of double membrane bodies (DMBs), considered to be FMV particles

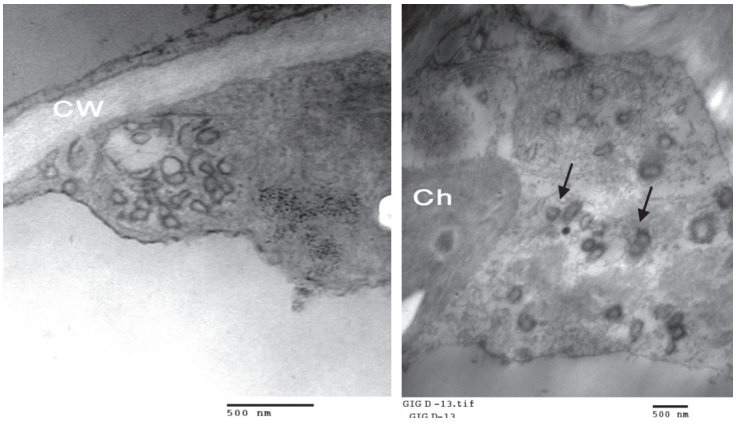


Figure 3. Groups of Double-membraned Bodies (DMB) in the cytoplasm of mesophyll cells from a naturally infected fig. Arrows indicate the aggregates of convoluted electron-dense double membrane elements. Ch, chloroplast, CW, cell wall, Bar = 500 nm.

with dimensions of 50–80 nm (Figure 3). Such structures were not found in PCR-negative samples to FMV, thus confirming previous reports on the aetiology of DMB in mosaic affected figs (Elbeaino *et al*., 2009b). These structures are a consistent feature of diseased figs, regardless of the variety and the country of origin (Plavsic and Milicic 1980; Martelli *et al*., 1993; Appiano *et al*., 1995; Castellano *et al*., 2007; Elbeaino *et al*., 2009a, 2009b).

The outcome of this preliminary work extends the knowledge on the spread of fig viruses in the Mediterranean region, particularly in Egypt for which no information was previously available. This is the first report of FLMaV-1, FLMaV-2, FMMaV and FMV occurring in the Egyptian fig orchards. Although this assessment was limited to 60 trees, the results obtained clearly indicate how the sanitary status of fig crop has deteriorated in Egypt (90% of viral infection). Particularly worrying is the incidence of FMV, since this has proved to be the unique virus closely correlated with the FMD (Elbeaino *et al*., 2009a). High incidence of FMV is not surprising considering the way this virus spreads in figs through infected propagating material (cuttings and grafting), and natural vectors (eriophyid mites). In Egypt there is no information on the presence of *Aceria ficus* (Eriophyidae) and *Planococcus ficus* (*Pseudococcidae*), the recognized vectors of FMV and FLMaV-2, respectively. However, such presence in the Egyptian orchards would likely aggravate the sanitary status and the level of infections in the surveyed areas.

The several FMV-infected samples found in association with most of the mosaic symptoms in the field further confirms what was previously reported regarding the aetiology of FMV. Nevertheless, this postulate was infringed by a few cases where FMV was detected in symptomless fig trees. Whether this is due to the virus strain present in the country or to the biological response of some Egyptian fig varieties to FMV infection remains to be determined.

The knowledge we have gained on the incidence of virus diseases of fig in Egypt provides information on which a sanitary selection, sanitation and certification programs can be initiated for the production of healthy propagating plant material of fig in this country.

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