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The Prevalence of Three Viruses Infecting Fig in Southern Turkey

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Abstract

In a survey of four fig-growing provinces of Turkey (Adana, Hatay, Şanlıurfa and Mersin), 132 samples were tested by reverse transcription-polymerase chain reaction (RT-PCR) to assess the presence of *Fig leaf mottle-associated virus 1* (FLMaV-1), *Fig leaf mottle-associated virus 2* (FLMaV-2) and *Fig mosaic virus* (FMV). All samples were negative in PCR to FLMaV-1, whereas FLMaV-2 and FMV were detected in six (4.5%) and 10 (7.6%) of the samples, respectively. Both viruses were found in fig trees collected in the provinces of Adana, Hatay and Şanlıurfa, but no infection was found in Mersin province. Sequence analysis of amplified DNA showed a level of nucleotides variability ranging from 1 to 10% for FLMaV-2 and from 6 to 17% for FMV isolates. Phylogenetic analysis grouped the Turkish FLMaV-2 isolates in one cluster together with other near-eastern FLMaV-2 isolates previously reported in GenBank.

Introduction

More than 10 million fig (*Ficus carica* L.) trees are grown in Turkey on a surface area of 474 492 ha (Anon., 2008). According to FAO, Turkey is the largest fig-producer in the world (285 000 tons) followed by Egypt (170 000 tons) and other Mediterranean countries (Anon., 2005). Contrary to other Mediterranean countries, in Turkey, figs are mostly cultivated in specialized orchards, and rarely as individual trees in outdoor gardens. Traditionally, fig plants are propagated through grafting and/or self-rooted cuttings, two techniques that favour the spread of pathogens, especially of viral diseases. Several viruses of different families (*Closteroviridae*, *Bunyaviridae*, *Flexiviridae*, *Partitiviridae* and *Caulimoviridae*) are reported to infect figs (Elbeaino et al. 2006, 2007, 2009a, 2010; Gattoni et al. 2009; Tzanetakis and Martin 2009; Walia et al. 2009). Despite the recent report of fig

mosaic disease (FMD) in fig orchards in Turkey (Çağlayan et al. 2009), the sanitary status of fig crop in the country is unknown. Accordingly, a preliminary investigation was initiated in four Turkish provinces (Adana, Mersin, Hatay and Şanlıurfa) in the south Turkey, where c. 4.0% of the national fig production is concentrated, to assess the presence of three fig-infecting viruses, namely *Fig leaf mottle-associated virus 1* (FLMaV-1), *Fig leaf mottle-associated virus 2* (FLMaV-2) and *Fig mosaic virus* (FMV) by using molecular assays (RT-PCR).

Material and Methods

A total of 132 fig samples were collected in May and September 2009. Samples were collected from both symptomatic (light yellowing, chlorotic mottling, leaf malformations) and symptomless trees; 45, 37, 20 and 30 samples, respectively, were collected from Adana, Mersin, Hatay and Şanlıurfa provinces. Approximately 22.7% of samples were from cv. Keten Köyneği, 35.3% from cv. Sarılop, 17% from cv. Göklop, 10% from cv. Bardakçı, while 15% were from unknown cultivars.

Thirty-five fig PCR-negative samples (c. 25% of the total), of which 15 originated from symptomatic trees, were all used for mechanical inoculation of a range of herbaceous hosts. Leaf tissue was ground in a sterile mortar with 0.05 M phosphate buffer containing 2.5% nicotine and rubbed onto carborundum powder-dusted leaves of *Chenopodium amaranticolor*, *C. quinoa*, *Nicotiana benthamiana*, *N. cavicola*, *N. occidentalis*, *Gomphrena globosa* and *Cucumis sativus*. Plants were kept in a glasshouse at 24–26°C for 2 weeks and observed for symptoms.

Total nucleic acids (TNAs) were extracted from 100 mg of leaf veins or cortical scrapings as described by Foissac et al. (2001). RT-PCR was performed using three sets of virus-specific primers designed by

Elbeaino et al. (2006, 2007, 2009b) for FLMaV-1, FLMaV-2 and FMV detection. One microlitre of PCR products was ligated to StrataClone™ PCR Cloning vector pSC-A (Stratagene, La Jolla, CA, USA) and subcloned into *Escherichia coli* SoloPACK cells. All plasmids containing DNA fragments were subjected to automated sequencing (ABI3730; Applied Bio., Sentromer DNA Teknolojileri Ltd. Co., Adana, Turkey). Nucleotide sequence analysis and construction of a phylogenetic tree were assisted with the DNA STRIDER 1.1 program (Marck 1988), CLUSTALX 1.8 (Pearson and Lipman 1988) and the NJPLOT package (Perrière and Gouy 1996), respectively.

Results

No virus was mechanically transmitted to any of the herbaceous test plant species, and all inoculated plants gave negative reactions in PCR to FLMaV-1, FLMaV-2 and FMV. Conversely, of 132 samples tested, FLMaV-2 was detected in 6 (4.5%) and FMV in 10 (7.6%) samples (Table 1; Fig. 1). All FMV PCR-positive samples originated from fig mosaic-diseased plants, unlike FLMaV-2, thus PCR-positive samples were not from symptomatic trees. In particular, FLMaV-2 was present only in Adana and Hatay provinces, whereas FMV was detected in all provinces, except for Mersin (Table 1). Accordingly, samples from Adana and Hatay had a higher level of infection (c. 20%) than those of the other two provinces.

The alignment of nucleotide sequences of four FLMaV-2-Turkish isolates, named IDB (Acc. No. FN668733), L2I (Acc. No. FN666271), BKE (Acc. No. FN666270) and ESO (Acc. No. FN668734), with

the corresponding HSP70 gene sequence of the type member in GenBank (Acc. no: AM286422), showed a level of nucleotide variation ranging from 1 to 10%, whereas the maximum amino acid variability was 6%.

Similarly, the sequence alignment of five FMV-Turkish isolates, designated, respectively, AMC (Acc. No. FN666272), U3 (Acc. No. FN666275), ESA (Acc. No. FN666274), UH (Acc. No. FN666276) and CLI (Acc. No. FN666273), compared with the homologous gene of FMV (Acc. No: FM864225), showed nucleotide variation ranging from 6 to 17%, and an amino acid divergence of a maximum of 12%. Accordingly, the phylogenetic tree placed the Turkish isolates in two sub-clades, closely related to another clade containing Syrian (Sy) and Lebanese (Lib) isolates, but far from isolates from Albania and Algeria (Fig. 2). Due to the lack of molecular information, no phylogenetic tree was constructed for FMV and Turkish isolates.

Conclusion

This is the first report of FLMaV-2 and FMV occurring in figs in Turkey. The prevalence of FLMaV-2 was 12%, an incidence similar to that in other Mediterranean countries (Elbeaino et al. 2009b). By contrast, the occurrence of FMV in figs sampled in Turkey was surprisingly low in comparison with that of this virus and with FMD in fig orchards worldwide (Castellano et al. 2007; Elbeaino et al. 2009a; Martelli 2009). No information is available on the presence in Turkey of *Aceria ficus* (Eriophyidae) and *Planococcus ficus* (Pseudococcidae), vectors of FMV, and to a limited extent, FLMaV-2, respectively. The aetiology of these diseases remains undetermined with

Table 1

Incidence of *Fig leaf mottle-associated virus 2* (FLMaV-2) and *Fig mosaic virus* (FMV) infection in four fig-growing provinces of Turkey as determined by RT-PCR assays

Province	Tested trees	Infected trees		FLMaV-1		FLMaV-2		FMV	
	No.	No.	%	No.	%	No.	%	No.	%
Adana	45	8	17.8	0	0	4	8.9	4	8.9
Mersin	37	0	0	0	0	0	0	0	0
Hatay	20	4	20	0	0	2	10	2	10
Şanlıurfa	30	4	13	0	0	0	0	4	13
Total infection	132	16		0		6		10	
Mean infection rate			12.1		0		4.5		7.6

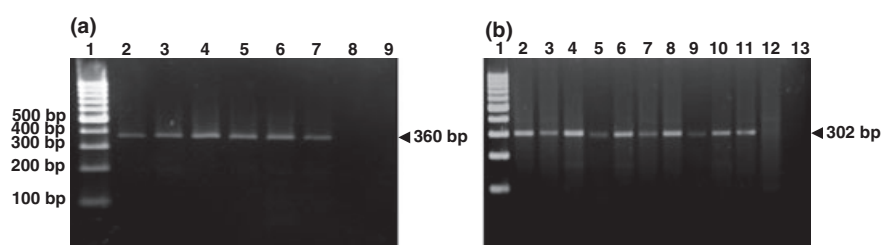
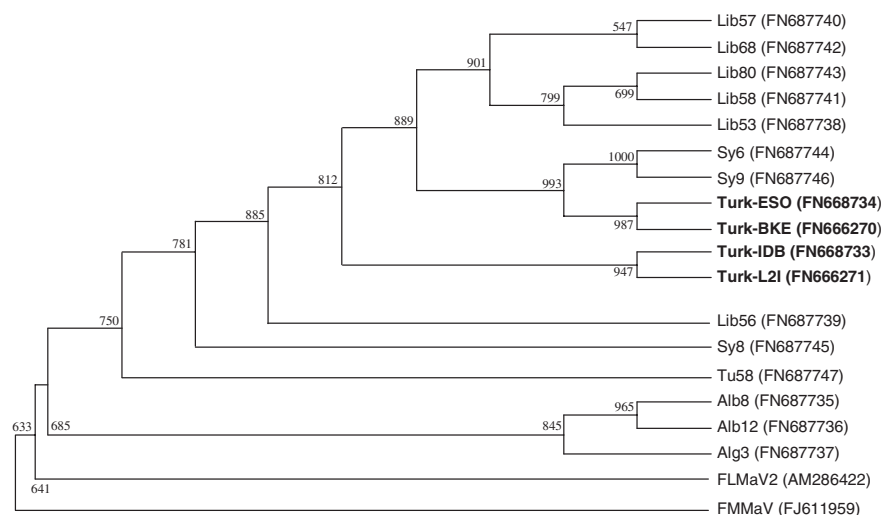


Fig. 1 Ethidium bromide-stained agarose gels showing the presence of *Fig leaf mottle-associated virus 2* (FLMaV-2) (1A) and *Fig mosaic virus* (FMV) (1B)-specific RT-PCR products. Lanes 2–7 (1A) and 2–11 (1B) represent RT-PCR-positive fig samples. Lanes 8–9 (1A) and 12–13 (1B) represent RT-PCR-negative fig samples. Lanes 1: 100-bp DNA Marker (Promega, Madison, WI, USA)

Fig. 2 Phylogenetic tree constructed with nucleotides sequences from the HSP70 gene of *Fig leaf mottle-associated virus 2* (FLMaV-2) isolates (Turk-ESO, Turk-BKE, Turk-IDB and Turk-L2I) from different provinces in Turkey. Accession numbers are reported between brackets. *Fig mild mottle-associated virus* (FMMaV) was used as an out-group species belonging to the *closterovirus* genus. Numbers are bootstrap values for 100 replicates



respect to the symptoms in the field and/or in fig PCR-negative trees. It is known that FMD coincides with the presence of FMV-infection, but FMD is also presumed to be induced by a complex of viruses and/or pathogens rather than by FMV alone (Elbeaino et al. 2009a). Fig culture is complicated because it is affected by a number of alterations (genetic disorders), mainly of fungal and bacterial origins (Mars 2003). This could explain the low incidence of FMD detected in Turkish fig orchards and the failure to mechanically transmit any virus from samples of symptomatic and/or symptomless trees to herbaceous hosts.

Although a small number of samples were tested, our failure to detect FLMaV-1 provides further evidence of the irregular distribution of this virus in Mediterranean countries, because it was also not detected in 50 samples in neighbouring Syria and in 15 samples in Algeria, but it was detected (c. 30%) in Tunisian and Lebanese fig samples (Elbeaino et al. 2009b). Our study extends knowledge on virus spread in fig crop in the Mediterranean region, particularly in Turkey for which only scanty information was previously available. Although our assessment was limited to 132 trees, it provides both an indication of the health status of Turkish orchards and the geographical distribution and prevalence of FLMaV-1, FLMaV-2 and FMV in Mediterranean countries.

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