

Sanitary selection of four virus-tested fig (*Ficus carica* L.) cultivars in Morocco

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Abstract

Fig (*Ficus carica* L.), a fruit tree widely grown in Morocco, is affected to a very large extent by fig mosaic disease (FMD), a complex with which eight viruses of different taxonomic position are associated. In order to select virus-free fig cultivars in Morocco, field surveys were conducted, between 2014 and 2018, in 11 different commercial orchards located in four regions (Azilal, El Jadida, Moulay Driss Zarhouné and Taounate). A total of 44 leaf samples representing 11 fig cultivars, without FMD symptoms, were prospected and collected for laboratory analyses. Total nucleic acids were extracted from leaf veins and tested by PCR for the presence of FMV, FLMaV-1, FLMaV-2, FMaV, FLV-1, FBV-1, FFkaV and FCrV using specific primers. Among the 11 tested cultivars, only four (Chetoui, Rhoudane, Elquoti Lebied and Embar Lekhel) were free from the tested viruses. This study allowed the identification of at least one “virus-tested” candidate clone from four different fig cultivars which can represent the potential mother plants for propagating materials in order to establish new fig nurseries and orchards.

Keywords: Fig, sanitary selection, viruses, Morocco

INTRODUCTION

Fig (*Ficus carica* L.), a fruit tree widely grown in Morocco, is an important crop for the country, both economically and socially. In Morocco, fig trees are widely cultivated throughout the country, mainly as individual trees in private gardens and orchards for family consumption, but more rarely in specialized plantations. Fig plantations cover a total acreage of about 55,000 Ha, with an annual production reaching 127,000 Tons (Anonymous, 2017). The most important fig production areas are Taounate (22,230 Ha), Chefchaouen (7,050 Ha), Al Hoceima (5,000 Ha), Ouazzane (3,150 Ha) and Tetouan (2,000 Ha). Other plantations are distributed among Taza, Nador, Essaouira, El Jadida, Safi and other regions. Although more than 24 cultivars of fig are produced in the country, the most popular are El Messari or Homrame or Johri, Lembdar Labied, Lembdar Lekhel, Rhoudane, El Koté and Aounq Hmam.

As with any commercial crop, fig trees are subjected to various pest problems including virus diseases. Fig mosaic disease (FMD) remains one of the most serious pathological problem facing fig germplasm exchange and production. This disease, first reported from California (Condit and Horne, 1933), is now known to have a worldwide distribution. FMD is a complex with which eight viruses of different taxonomic position are associated: 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 (FMaV), Fig fleck-associated virus (FFkaV), Fig latent virus 1 (FLV-1), Fig cryptic virus (FCrV) and

Fig badnavirus 1 (FBV-1) (Martelli, 2011). FMV is the agent that occurs in symptomatic plants more often than any of the other fig-infecting RNA viruses and is the major incitant of mosaic (Chiumenti *et al.*, 2013). Although no estimates of the economic impact of FMD are available, the notion that severely affected trees are less productive than those with milder symptoms and suffer premature fruit abscission has been taken as an indication that FMD can have a detrimental effect on the crop (Chiumenti *et al.*, 2013).

The only known cure against virus and virus-like diseases is to provide healthy (virus-free) planting material, usually within the framework of a phytosanitation program based on diagnosis, detection and elimination of the causal agent(s) and the maintenance and distribution of healthy stocks. In this regard, the National Office for Food Safety (ONSSA) has launched, since 2013, a certification scheme for the production of fig plants in Morocco. This scheme has the aim of providing plants that are true-to-type, free from two fig-infecting viruses (FLMaV-1 and FLMaV-2), and substantially free from other pests.

The objective of the present study was to identify FMD-free fig trees to be used as mother plants for the production of fig plants in Morocco in order to establish new certified fig nurseries and orchards. To this aim, RT-PCR analysis has been used to detect the aforementioned viruses in candidate mother plants (without FMD symptoms), which were collected from different regions during field surveys along the country.

MATERIAL AND METHODS

Field surveys were conducted, between 2014 and 2018, in 11 different commercial orchards located in four fig-growing regions: Azilal, El Jadida, Moulay Driss Zarhounne and Taounate. A total of 44 leaf samples representing 11 fig cultivars (Table 1), without FMD symptoms, were prospected and collected for laboratory analyses. Total nucleic acids were extracted from leaf veins using the RNeasy Plant Mini Kit (Qiagen, Germany) and tested for the presence of FMV, FLMaV-1, FLMaV-2, FMMAV, FLV-1, FBV-1, FFkaV and FCrV using specific primers (Table 2). For RNA viruses (FLMaV-1, FLMaV-2, FMMAV, FLV-1, FFkaV and FCrV) 0.5 µg TNA were denatured by boiling at 95°C for 5 min, then reverse-transcribed with random primers and M-MLV reverse transcriptase (Life Technologies, USA) for 1 h at 42°C (Minafra and Hadidi, 1994). PCR amplification for FBV-1 was done directly on total nucleic acid extracts without reverse transcription. Amplification products were analyzed in 1.2% agarose gel electrophoresis. Gels

were electrophoresed in Tris-acetate running buffer, pH 8, at 100 volt for 1 hour. Amplified products were seen under UV light after staining with Ethidium Bromide.

RESULTS AND DISCUSSION

RT-PCR result showed that of the 44 samples tested, 39 (88.6%) were infected by at least one of the eight tested viruses, while the incidence of mixed infections was over 68% (30 of 44 trees). FLMaV-1 and FMV were the prevailing viruses with infection rates of 56.9% and 43.2%, respectively, followed by FMMAV (31.8%), FLV-1 (25%), FLMaV-2 (20.4%) and FFkaV (6.8%). FBV-1 and FCrV were not found. Similar results were found by Elbeaino *et al.* (2011c) in the Canary Islands (Spain) where FLMaV-1 and FMV were the prevailing viruses with infection rates of 53.3% and 50%, respectively, followed by FMMAV (43.3%), FLV-1 (33.3%) and FLMaV-2 (33.3%). All trees sampled did not show apparent FMD-like symptoms at the time of the survey, among them 25 were FMV-negative, whereas 19

Table 1: List of the sampled fig cultivars without FMD symptoms, from commercial orchards, to be assayed by RT-PCR against eight viruses associated with FMD

Region	Sampled cultivars	Total number of sampled trees
Azilal	Chetoui	4
	Elquoti Lebied	4
	Rhoudane	
El Jadida	Nabout	4
	Embar Lekhel	4
	M'tioui	
Moulay Driss Zarhounne	Bousbati	4
	Embar Lekhel	4
Taounate	Jebliia	4
	El Messari	4
	Lembdar Lebied	4
	Lembdar Lekhel	4
	Aounq	4
Total	11	44

Table 2: PCR primers used in the present study

Virus species	Primer	Sequence (5'-3')	Amplicon size (bp)	References
FMV	BB42 up	TGGCAGATTC AAGGATAATGG	218 bp	Elbeaino <i>et al.</i> , 2009
	BB42 down	TGGGACATTC TTTGTGTCAGG		
FLMaV-1	N17s	CGTGGCTGATGCAAAGTTTA	350 bp	Elbeaino <i>et al.</i> , 2006
	N17a	GTAAACGCATGCTTCCATGA		
FLMaV-2	F3s	GAACAGTGCCATCAGTTTGATTTG	360 bp	Elbeaino <i>et al.</i> , 2007
	F3a	CCCACCTCCTGCGAAGCTAGAGAA		
FMMAV	LM3s	AAGGGGAATCTACAAGGGTCG	311 bp	Elbeaino <i>et al.</i> , 2010
	LM3a	TATTACGCGCTTGAGGATTCG		
FLV-1	FFup	CGCTTTGCCCAATGTGCAGAT	125 bp	Gattoni <i>et al.</i> , 2009; Modified by Chiumenti <i>et al.</i> , 2013
	FFrev25	TARTCDGATTCHACRCACAGGTC		
FBV-1	P1-s	GCTGATCACAAGAGGCATGA	214 bp	Minafra <i>et al.</i> , 2012
	P1-as	TCCTTGTTTCCACGTTCCCTT		
FFkaV	d8-s	ATGACGACTGTCAACTCCCT	270	Elbeaino <i>et al.</i> , 2011b
	d8-a	TAAAGCCAGGGTGGGAGTGTTG		
FCrV	R1-s	TCGATTGTCTTTGGAGAGG	353	Elbeaino <i>et al.</i> , 2011a
	R1-a	CGCATCCACAGTATCCCAT		

(43.2%) were FMV-positive, thus confirming the high level of the association between FMD and FMV (Martelli, 2011). During a preliminary survey in the Canary Islands in autumn of 2009, Elbeaino *et al.* (2011c) found that among 12 trees that did not show apparent FMD-like symptoms at the time of the survey, eight were FMV-negative; whereas 15 (88%) of the 17 symptomatic plants were FMV-positive.

Five virus-free trees were found during the survey, two plants of cv. Chetoui and one of each cvs. Rhoudane, Elquoti Lebied and Embar Lekhel. These plants may represent potential sources of material for propagation in the framework of the certification scheme for the production of fig plants, which has been already established since 2013 in Morocco.

CONCLUSION

The present work has shown a much deteriorated sanitary status of the fig crop in Morocco (88.6% 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. As there are no chemical products against these agents, the only known cure is to provide virus-free planting material, usually within the framework of a phytosanitation program based on diagnosis, detection and elimination of the causal agent(s) through sanitation. The present study allowed selecting four virus-tested cultivars that could be considered as potential candidate mother plants for the national certification program for the production of fig plants, which has been already established since 2013 in the country. Sanitary selection is a crucial step for virus control but it is also important to take into consideration the epidemiology of the detected viruses which is essential to preserve the selected fig plants. Further studies are planned in the country to initiate sanitation through heat therapy, meristem tip culture *in vitro* and combination of both techniques for obtaining virus-free mother plants for cultivars that are infected with viruses associated with FMD.

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