Journal of Integrative Agriculture 2017, 16(6): 1417–1421

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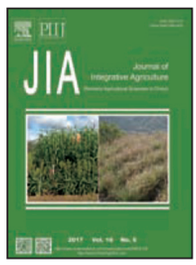

SHORT COMMUNICATION

Analysis of fig tree virus type and distribution in China

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Abstract

The common fig (*Ficus carica* L.) was one of the earliest horticultural crops to be domesticated. A number of different viruses can infect fig trees including *Fig mosaic virus* (FMV) that has been detected in several commercial fig trees in Xinjiang, China. However, the distribution of FMV and other fig-infecting viruses in China remains unknown. In the present study, a sample from an ancient fig tree growing in Xinjiang was investigated by electron microscopy (EM) followed by PCR/RT-PCR, and FMV, *Fig badnavirus* 1 (FBV-1) and Fig leaf mottle-associated virus 1 (FLMaV-1) were detected. Fig leaf samples (252) from commercial orchards across China were subjected to PCR/RT-PCR, and FMV, FBV-1 and Fig fleck-associated virus (FFkaV) were relatively abundant (44.4, 48.4 and 44%, respectively), while FLMaV-1 and Fig mild mottle-associated virus (FMMaV) were much scarcer (5.6 and 0.4%, respectively), and FLMaV-2, Fig cryptic virus (FCV), and Fig latent virus (FLV) were not detected. The presence of disease-causing viruses in fig trees presents a significant challenge for fig producers in China. This study may help to promote actions aimed at controlling fig viruses, especially FMV.

Keywords: *Ficus carica*, fig mosaic disease, fig-infecting viruses, field survey, molecular detection

1. Introduction

The common fig (*Ficus carica* L., Moraceae) was one of the first fruit crops to be domesticated during the birth of old world horticulture in the Mediterranean Basin and Southwest Asia (Zohary *et al.* 2012). A number of viruses have been identified in fig trees and classified definitively or tentatively as members of the families/genus *Emaravirus* (*Fig mosaic virus*, FMV), *Closteroviridae* (Fig leaf mottle-associated virus 1 and 2, FLMaV-1 and 2, and Fig mild mottle-associated virus, FMMaV), *Caulimoviridae* (*Fig badnavirus* 1, FBV-1), *Betaflexiviridae* (Fig latent virus 1, FLV-1), *Partitiviridae* (Fig cryptic virus, FCV) and *Tymoviridae* (Fig fleck-associated virus, FFkaV). Although FMV is consistently associated with fig mosaic disease (FMD) (Elbeaino *et al.* 2009; Walia *et al.* 2009), fulfillment of Koch's postulates has yet to be done.

FMD was originally recorded in California (Condit and Horne 1933) and is now found in almost all fig orchards worldwide (Martelli 2011). Symptoms of this disease vary widely from typical discoloration to distortion of the leaves. Severe foliar symptoms may be accompanied by stunted growth and reduced productivity. FMD can therefore result

Received 2 August, 2016 Accepted 4 December, 2016
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doi: 10.1016/S2095-3119(16)61551-4

in significant economic losses (Stover *et al.* 2007). The disease-causing agent is transmitted by the eriophyid mite *Aceria ficus* with semi-persistent modality or *via* grafting (Flock and Wallace 1955). Cytopathology studies of FMD show that parenchyma cells of diseased plants consistently contain double-membrane bodies (DMBs) 90–200 nm in diameter (Martelli *et al.* 1993), and these are also transmitted by *Aceria ficus*.

Fig trees were introduced into China during the Han Dynasty (206 BC–220 AD) and are now cultivated widely throughout the country. Although FMD has been reported in China (Pittman 1935), a survey performed in June 2006 failed to discover the disease in the germplasm repository of the Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences, or in commercial orchards in Shandong Province of China. Recently, however, we observed FMD in several orchards in Xinjiang, and found that most symptomatic samples were infected with FMV (Mijit *et al.* 2015). Despite this, the distribution of FMV and the occurrence of other viruses naturally infecting fig trees in China remain unknown. In this study, a large-scale survey was carried out to detect the main viruses infecting fig trees in China.

2. Materials and methods

Samples were collected from commercial orchards in Xinjiang between 2013 and 2015. Orchards in Beijing and Jiangsu were also included. Leaf samples, in particular symptomatic leaves from fig trees infected with FMD, were randomly collected for virus detection (Table 1). At least three young leaves from different spatial positions on a single tree were collected and combined as one sample. Young leaves from an old FMD-infected fig tree (about 160 years old) growing in Kashgar were also taken to the Center of Analysis and Measurement, Zhejiang University, for electron microscopy (EM) observations.

Thin sections were prepared for EM from tissue fragments excised from young leaves of the 160-year-old tree. Ultrathin sections were then stabilized and stained according to standard procedures (Martelli and Russo 1984). Validation of viruses in old fig tree samples was performed by PCR/RT-PCR as described below.

All samples were tested by PCR/RT-PCR for identification of viral infections. Total nucleic acids used for direct PCR detection were purified as described previously (Laney *et al.* 2012). Total RNA was extracted using TRIzol reagent (Tiangen Biotech, Beijing) according to the manufacturer's protocol, and cDNA was synthesized using M-MLV reverse transcriptase (Promega, Shanghai) by PCR with 2× *Taq* mastermix (Tiangen Biotech). Primers used for amplification of FMV, FFkaV, FBV-1, FLMaV-1 and 2, FMMaV, FCV, and FLV-1, together with cycling parameters, were as described previously (Elbeaino *et al.* 2006, 2007, 2009, 2010, 2011; Gattoni *et al.* 2009; Tzanetakis *et al.* 2009; Agha *et al.* 2013) and details are included in Appendix A. Selected amplified products were then sequenced directly.

3. Results and discussion

FMD was detected in Xinjiang, but not in Beijing or Jiangsu, and in almost all commercial orchards in Xinjiang, especially those in Atushi (Fig. 1). In several orchards severely affected by FMD, nearly all fig trees were diseased, and a range of leaf symptoms were visible including discoloration of young leaves at the tops of trees, with some showing chlorotic mosaic (Fig. 1-A–C, H), mottle-necrotic ringspots (Fig. 1-D) and severe mosaic symptoms (Fig. 1-G). In some leaves, malformation and vein-cleaning were also obvious (Fig. 1-E–F), and some diseased trees were also reduced in size.

In addition to commercially planted fig trees, Xinjiang also has the largest number of preserved old fig trees (~100–500 years old) in China, and while most were found to be growing vigorously and large in size, some showed typical symptoms of FMD. In order to verify viral infection, a symptomatic old fig tree (~160 years old) growing in Kashgar was subjected to EM. Filamentous virus-like particles (Fig. 1-I) and typical DMBs (Fig. 1-J) were observed in the cytoplasm of mesophyll cells. Filamentous virions were flexuous and clustered in bundles possibly associated with closterovirus infection. These results indicated the presence of FMV and *Closteroviridae* family viruses in this old fig tree.

Three viruses in the family *Closteroviridae*, FLMaV-1 and 2 and FMMaV, have been identified in fig (Elbeaino *et al.* 2006, 2007, 2010). Tissue from the old fig tree was subjected to RT-PCR, and the result revealed infection with FMV and FLMaV-1. In addition to these two viruses, FBV-1 was also detected in this old fig tree by PCR.

Large-scale investigation of fig-infecting viruses was performed on the collected samples by PCR/RT-PCR, and FBV-1 (48.4%), FMV (44.4%), FFkaV (44%), FLMaV-1 (5.6%) and FMMaV (0.4%) were detected, but FLMaV-2, FCV and FLV-1 were absent (Table 1). Several amplified products from each virus were sequenced directly and the resultant sequences used in BLAST searches (Appendix B). Chinese isolates of FMV, FLMaV-1, and FMMaV shared 92–97% nucleotide sequence identity with those isolated from Montenegro, and Bosnia and Herzegovina. Two different sequences from FBV-1 isolates from Atushi, Kashgar, Beijing, and Jiangsu were identical to those from Iran (KU821726) and USA (JN050864). In the case of FFkaV, sequences of isolates