New viruses found in fig exhibiting mosaic symptoms

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Abstract

Mosaic is the most widespread viral disease of fig, affecting the crop wherever it is grown. The causal agent of the disease was poorly characterized and until recently it was considered a virus-like agent with double membrane bound semispherical bodies transmitted by eriophyid mites. During the molecular characterization of the Fig mosaic virus we discovered two new closteroviruses and a new badnavirus affecting the tree used in our studies. The characterization and presence of the three new viruses in mosaic-affected plants is the subject of this communication.

Keywords: Fig mosaic, Emaravirus, Closterovirus, Badnavirus

Introduction

Fig mosaic (FM) was first discovered in 1933 [4] and has since been found worldwide. The symptoms vary from tree to tree ranging from mild mosaic and ringspots to malformation of leaves and tree decline. Double membrane-bound bodies have been found associated with FM [1, 3], and recently a virus in the genus *Emaravirus*, Fig mosaic virus, was proven to be the causal agent of the disease [7, 8, 13]. Other than FMV several other viruses have been found in FM trees, including clostero-, umbra-, luteo- and cryptic viruses [5, 6, 13]. These discoveries and the symptom range suggested that FM may be caused by the synergistic effects of several viruses when they co-infect the crop. In the plant used in our study we discovered three new viruses, two closteroviruses, provisionally named Arkansas fig closterovirus-1 and -2 (AFCV-1 and 2) and one in the badnavirus named Fig badnavirus-1 (FBV-1). These three viruses are the focus of this communication.

Materials and methods

Mechanical transmission: At least eight plants of the following potential alternative virus hosts species Nicotiana occidentalis, N. tabacum, N. sylvestris, N. rustica, N. benthamiana, *Gomphrena globosa*, Glycine max, Cucumis sativus, Pisum sativum, Vigna unguiculata, Phaseolus vulgaris var. Black Valentine, Spinacia oleracea, Chenopodium *amaranticolor*, and *C. quinoa*, were inoculated with symptomatic fig tissue ground in cold 0.05 M phosphate buffer pH: 7.0. The plants were kept in an insect-free greenhouse under 14 h light/10 h dark regime, then observed for visual symptoms for one month followed by reverse transcription polymerase chain reaction (RT-PCR) tests for each of the three viruses communicated in this report.

DsRNA extraction: The Yoshikawa and Converse [14] and Tzanetakis and Martin [11] dsRNA extraction methods were used on tissue of a severely FM affected fig plant. Only symptomatic tissue was used in the extraction. In the final step of the extraction, nucleic acids were sequestered on glass in the presence of 50 % EtOH/50 % TE solution as described in Tzanetakis et al. [12] and eluted in 100µl TE after 10 min incubation at 70C.

Sequencing: cDNA synthesis was performed using Superscript III reverse transcriptase and a primer with a 6-nucleotide random region and a 16 nt known sequencing, making it essentially a random reverse transcription with the incorporation of known sequence that could be used downstream in a DOP-PCR amplification. After incubation at 50C for 60 min, the reaction was terminated by incubation at 75C for 15 min. The reaction was then digested by RNase H and subjected to PCR using a primer with identical sequence to the known portion of the reverse transcription primer and using 1/10 (v/v) RT reaction in the PCR. The PCR product was either shotgun cloned using TOPO technology (Invitrogen) followed by Sanger sequencing at the University of Arkansas DNA resource center or subjected to Illumina sequencing as described by the manufacturer at the Central Services Laboratory of Oregon State University.

Sequence and phylogenetic analysis: Contigs of Illumina 36 bp single end reads were first constructed using Velvet, Edena, QSRA, SSAKE, and VCAKE algorithms. CodonCode Aligner 3.0 (CodonCode Corporation, Dedham, MA) was then utilized to assemble these five sets of contigs into larger contigs. Those contigs along with sequences obtained through Sanger sequencing were compared with database sequences using BLAST [2]. Regions that had similarities with described viruses were used to develop primers for detection of the new viruses and for closing the sequence gaps that were not obtained through sequencing. Phylogenetic analysis was performed with ClustalW [10] using the neighbor-joining algorithm, Kimura's correction and bootstrap consisting of 1000 pseudoreplicates.

Detection: Primers AFCV1F (5'-CTGTATCTGTCATTACCTCTTCGGG) and
AFCV1R (5'-ATGCTTCCTCGGCTGC);
AFCV2F (5'-GTTCGGAATTAGTTAATAGATACGGTC) and
AFCV2R (5'-ACCCGCTAGAGTAATCAGTCAAAGTT);
FBVF (5'-ACCAGACGGAGGGAAGAAAT) and
FBVR (5'-TCCTTGCCATCGGTTATCTC);
FLMaV-1F (5'-GGGTTGGAGTTCGGG) and
FLMaV-1R (5'-ACGTGTCTGATGGGGA);
FLMaV-2F (5'-ACTTGCGACCCGATAA) and
FLMaV-2R (5'-CCTAGTCGGAGTGGATT)

that amplify a 375, 1671, 474, 580 and 479 base region of the genomes AFCV-1, -2 and FBV-1, Fig leaf mottle associated virus -1 and -2 (FLMaV-1 and -2) respectively were selected for RT-PCR detection. The primers were used on the tree of our study and another 39 FM accessions provided by the fig germplasm repository in Davis, California. The PCR program on an Eppendroff Mastercycler® using Genescript Taq polymerase consisted of denaturation for 2 min a 94C followed by 40 cycles at 94C, 55 C and 72C for 30, 10 and 90 sec respectively and terminated with 10 min incubation at 72C. Several amplicons from each virus were sequenced and all were virus specific, validating the detection protocols.

Results and discussion

Ds RNA extractions from an Arkansas FM tree revealed the presence of several bands ranging from more than ten to less than one kilobase (Figure 1). Shotgun cloning and Illumina sequencing revealed the presence of at least four viruses in the FM tree used in our study. The Arkansas FMV isolate is less than 90 % identical at the nucleotide level (97 % aa identity) to the sequenced European isolate. The AFCV-1 and -2 belong to the *Closterovirus* genus and are more closely related to Fig leaf mottle associated virus-1 than any virus found in the database. The Badnavirus is most closely related to *Citrus yellow mosaic virus* and *Cacao swollen shot virus* (Figure 2).

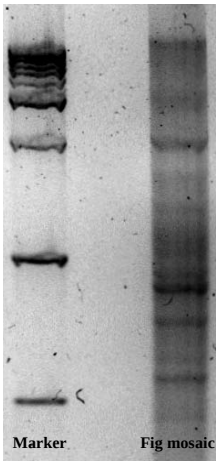

Fig. 1. Double stranded RNA (dsRNA) extraction from fig mosaic diseased plant. Left: 1 kilobase ladder, top band: 10 kilobases, bottom band: 0.5 kilobases; Right: Fig dsRNA.

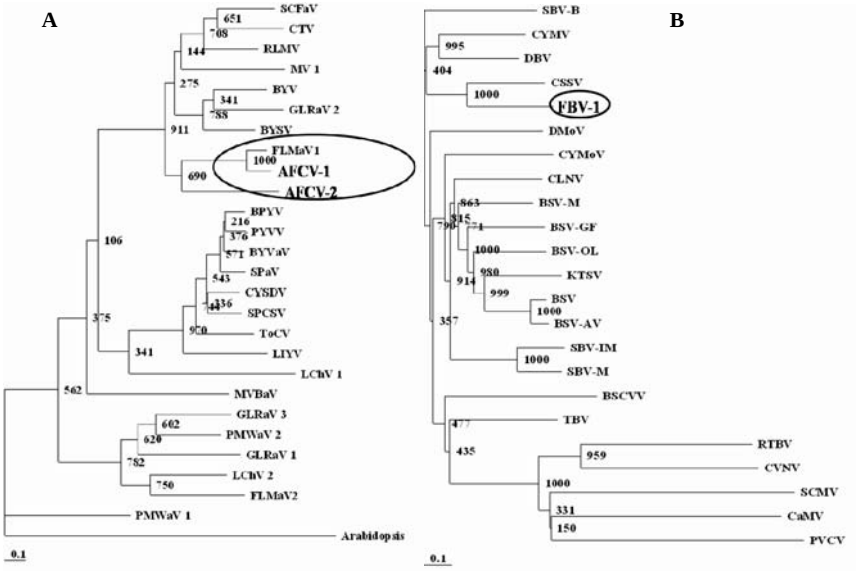

Fig. 2 Phylogenetic analysis of Arkansas fig closteroviruses (A) and Fig badnavirus (B). Bootstrap values presented on the nodes of the trees.

Our survey revealed that AFCV-1 is not a widespread virus in FM germplasm accession unlike AFCV-2 and FBV-1 that were present in several symptomatic trees (Figure 3). The similarity of AFCV-1 to FLMaV-1 led us investigate the possibility that the two virus are actually the same species. None of the 40 plants tested were found infected by FLMaV-1 or -2. Another conclusion of the survey was the presence of the AFCV-2 vector in California given that there are accessions with one of the trees infected and others that are free of the virus, unless there were multiple plants of the same accession brought into the collection. Asymptomatic fig plants were also tested for the presence of the new viruses and one of them was found to be infected with FBV-1, indicating that the virus is asymptomatic on this fig accession.

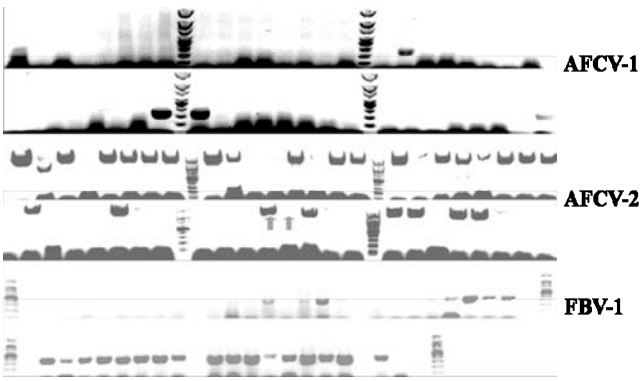

Fig. 3 Detection of Arkansas fig closteroviruses and Fig badnavirus in mosaic accessions from the fig repository in Davis, California. Arrows indicate two trees of a single accession, one infected and one free of Arkansas fig closterovirus-2.

Fourteen plant species of herbaceous indicators were tested for transmission of the three new viruses and *G. max* was found to be an alternative host for AFCV-2 and FBV-1 developing mild mottling symptoms. Although *N. occidentalis*, *N. tabacum*, *N. rustica*, *N. sylvestris*, and *P. sativum* did not develop symptoms, they all tested positive for FBV-1. All positive tests were verified by sequencing the PCR products.

A single FM trees was found infected with at least four viruses: FMV, AFCV-1 and -2 and FBV-1. The diversity of the viruses and their vectors reveal the complexity of the disease and the symptomatology observed in FM trees.

FMV belongs to the genus *Emaravirus* that includes viruses related to tospoviruses and is transmitted by the eriophyid mite *Aceria ficus*. Several closteroviruses belonging to the genera *Closterovirus* and *Ampelovirus*, presumably transmitted by aphids and mealybugs respectively, have been found in fig in Europe, North Africa, the Middle East, and the United States [9, 13]. Badnaviruses are vectored primarily by mealybugs and aphids and thus FBV-1 may share vectors with the closteroviruses that infect the crop.

FM is a disease that is now known to be caused by the emaravirus FMV. The FM symptom diversity suggest that the additional viruses that have been found in FM plants in both the Old and New World may play a significant role on the expression of the disease. It is possible that some of the newly identified viruses share vectors that may account, in addition to the clonal propagation, for the large numbers of viruses that fig harbors. We are working towards further characterization of these new viruses including symptoms in single infections and possible vector species.

Literature

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