Diseases Caused by Viruses

First Report of Citrus Virus A in Texas Associated with Oak Leaf Patterns in *Citrus sinensis*

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Plant Dis. 106:2005, 2022; published online as <https://doi.org/10.1094/PDIS-03-21-0628-PDN>. Accepted for publication 24 December 2021.

In 2018, two new negative sense coguviruses in citrus were identified, citrus concave gum-associated virus (CCGaV) and citrus virus A (CiVA) (Navarro et al. 2018a, b). Since then, members of the genus *Coguvirus* have also been detected in other plant species (Svanella-Dumas et al. 2019; Wright et al. 2018; Xin et al. 2017). In 2016, leaf flecking with oak leaf patterns was observed in five embryo-rescued navel orange (NO) (*Citrus sinensis* [L.] Osbeck) trees grafted on C22 (*C. sunki* × *Poncirus trifoliata*) rootstock that were maintained in a shade house. Madam Vinous (MV) sweet orange trees graft-inoculated with blind buds from the symptomatic NO plants developed the same symptoms in the new growth. These symptoms were similar to those on the citrus concave gum (CG) source tree of the California isolate CG301, one of the standard citrus disease isolates used as a positive control for biological indexing (Roistacher et al. 2000). None of the trees with oak leaf symptoms tested positive with reverse transcription (RT)-PCR for a panel of viruses and viroids commonly infecting citrus. In this study, CG301 leaf RNA-Seq data were used as a platform to identify any viral agents associated with the oak leaf symptoms observed in the symptomatic NO trees. Of ~162.8 million paired-end CG301 RNA-Seq reads (150 bp), the de novo assembly of ~9.6 million reads, not mapped to the *C. sinensis* genome (v.1.1), yielded 5,375 contigs. BLASTn using the NCBI virus database (txid 10239) identified two contigs, #49 (6,715 nt) and #20 (2,764 nt), which exhibited ~96% sequence identity to, respectively, RNA1 and 2 of the CiVA isolate W4 (MG764565; MG764566) and 71 to 73% identity to that of the CCGaV isolate CGW2 (KX960112; KX960111). A 5'-Nuclease assay based on contig #20 detected coguvirus sequences in the five symptomatic NO and graft-inoculated MV trees as well as in CG301, but not in 44 asymptomatic field trees located near the shade house where the symptomatic NO trees had been kept. A full genomic sequence of the

coguviruses present in CG301 and a symptomatic NO tree was reconstructed by RT-PCR. Both the CG301 and NO isolate have a 6689-nt long negative sense RNA1 (MT922052; MK689372) encoding RNA-dependent RNA polymerase (RdRp) and a 2739-nt long ambisense RNA2 (MT922053; MK689373) encoding movement protein (MP) and nucleocapsid protein (NP). CG301 and NO isolates share ~96% nucleotide sequence identity. The genomes of both CG301 and NO isolates share 95.4 to 97.8% sequence identity to that of CiVA isolate W4 and 70 to 72.9% sequence identity to CCGaV isolate CGW2. BLASTp showed that RdRp of CG301 and NO isolates have 96.3 to 97.7% sequence identity to CiVA W4 RdRp and ~77% to CCGaV CGW2 RdRp. These data indicated the presence of CiVA in the symptomatic NO trees and in the concave gum source tree CG301. Recent reports of CiVA in South Africa and Greece indicated a potentially wider distribution of CiVA in various citrus growing regions that may be associated with two graft-transmissible citrus diseases, CG and impietratura disease (Beris et al. 2021; Bester et al. 2021; Roistacher et al. 2000; Velázquez et al. 2019). Although the source of CiVA in the symptomatic NO trees and the degree of CiVA prevalence in Texas has not been determined, a possible involvement of vectors or other means of spread (e.g., seed transmission) cannot be ruled out (Timmer et al. 2017). The current study demonstrated the need for further studies to determine the level of threat of coguviruses for citrus production in Texas.

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The author(s) declare no conflict of interest.

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Keywords: citrus concave gum-associated virus, citrus virus A, RNA virus, sweet orange

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