First Report of Cacao Mild Mosaic Virus Associated with Symptomatic Commercial Cacao (*Theobroma cacao*) Trees in Puerto Rico

A. S. Puig,^{1,†} R. Ramos-Sobrinho,² C. Keith,² N. Kitchen,² O. Gutierrez,¹ R. Goenaga,³ and J. K. Brown²

¹ USDA-ARS Subtropical Horticulture Research Station, Miami, FL 33158

² School of Plant Sciences, University of Arizona, Tucson, AZ 85721

³ USDA-ARS Tropical Agriculture Research Station, Mayaguez, PR 00680

Plant Dis. 104:3089, 2020; published online as <https://doi.org/10.1094/PDIS-04-20-0745-PDN>. Accepted for publication 11 May 2020.

The cacao industry is rapidly expanding in Puerto Rico (PR), where ~20,000 trees have been planted in the past few years (DAPR 2017). In September 2019, leaf chlorosis and purple mottling on unripe pods were observed on cacao trees (genotype RIM-52) on a commercial farm in PR. In December 2019, ripe pods with red spots were found on an additional RIM-52 tree on the same farm. Tissue sampled in September (one symptomatic leaf and one symptomatic pod from tree 1) and December (two symptomatic pods and two asymptomatic leaves from tree 2) was tested using recently developed molecular diagnostics for *Cacao mild mosaic virus* (CaMMV) and *Cacao yellow vein banding virus* (CYVBV), two badnaviruses from nearby Trinidad that cause symptoms similar to those observed here (Chingandu et al. 2017). Total DNA was isolated and used as template for polymerase chain reaction amplification of the movement protein and coat protein (MP-CP) domain of CaMMV and CYVBV using the primer pairs CaMMV-1112F, 5'-TACGGAGACTGTGACTCAACCA-3'/CaMMV-1959R, 5'-GTTTGGTAGGTTCTTGATCTGC-3', and CYVBV-1223F, 5'-AACGAGGACTACAACCTCAGGC-3'/CYVBV-2164R, 5'-TCCTCCAGTATCTCTTCATCCC-3', respectively. To enable species-level identification following amplification with the CaMMV diagnostic primers (no amplification was obtained with CYVBV primers), the RT-RNase H domain was amplified from a representative isolate (tree 1) using CaMMV-specific primers (CaMMV-RT-F, 5'-TGCGGATTAAGAAGGCAGTAG-3'/CaMMV-RT-R, 5'-GAGGTGATAGTAGGCGGTTTG-3'). Amplicons of expected sizes, ~900 bp (MP-CP) from leaf ($n = 1$) and pods ($n = 3$) and ~1,840 bp (RT-RNase H) from leaf ($n = 1$) and pod ($n = 1$), were cloned into the pGEM-T Easy plasmid vector and bidirectionally Sanger sequenced ($n = 2$ per amplicon). Pairwise comparisons of MP-CP and RT-RNase H sequences were done using the Sequence Demarcation Tool (SDT) version 1.2 (Muhire et al. 2014). The

MP-CP sequences ($n = 8$; GenBank numbers MT253655 to MT253658 and MT262888 to MT262891) shared 95.2 to 96.0% nucleotide identity with CaMMV (accession KX276640). Based on *Badnavirus* species demarcation criterion, of $\geq 80\%$ nucleotide identity for the RT-RNase H domain (Geering and Hull 2012), the PR (accessions MT253659 to MT253662) and Trinidad (accession KX276640) isolates are the same species, CaMMV, with 95.9 to 96.3% nucleotide identity. Variability among PR isolates ranged from 98.7 to 99.4% sequence identity between trees and 99.3 to 99.5% within the same tree. A Bayesian phylogenetic tree was reconstructed using concatenated MP-CP and RT-RNase H sequences (CIPRES portal; Miller et al. 2010) with the evolutionary model GTR+I+G predicted using MrModeltest version 2 (Nylander 2004). The phylogenetic tree supported the results obtained from SDT comparisons, grouping the PR isolates with the CaMMV isolate from Trinidad (study S26001 in TreeBASE). CaMMV, previously known as cacao Trinidad virus A, was reported in 1943 (Posnette 1944) and associated with 7 to 33% yield reduction (Cope 1953; Swarbrick 1961). This is the first report of CaMMV in PR and believed to be the first report outside of Trinidad. This discovery is significant to the global cacao industry because it indicates CaMMV is more widespread than originally thought and highlights the importance of molecularly indexing germplasm before releasing from quarantine. Additional studies are needed to determine the distribution of CaMMV in PR and the Americas as well as its impact on production.

References:

- Chingandu, N., et al. 2017. Arch. Virol. 162:1363.
- Cope, F. W. 1953. Page 126 in: Report on Cacao Research, 1945-1951. Imperial College of Tropical Agriculture, Trinidad.
- Department of Agriculture of Puerto Rico (DAPR). 2017. Final Performance Report, FY 2013. <https://www.ams.usda.gov/sites/default/files/media/PR13SCBGPFinalReport.pdf>
- Geering, A. D. W., and Hull, R. 2012. Page 429 in: Virus Taxonomy: 9th Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, London, U.K.
- Miller, M. A., et al. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. 2010 Gateway Computing Environments Workshop.
- Muhire, B. M., et al. 2014. PLoS One 9:e108277.
- Nylander, J. A. A. 2004. MrModeltest version 2. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.
- Posnette, A. F. 1944. Trop. Agric. 21:105.
- Swarbrick, J. T. 1961. Trop. Agric. 38:245.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: cacao Trinidad virus, CTV, *Caulimoviridae*, *Badnavirus*, emerging virus, mealybug vector, tropical tree, CaMMV

[†]Indicates the corresponding author.
E-mail: alina.puig@usda.gov (A.S.P.)