First Report of East African Cassava Mosaic Virus-Uganda Infecting the Nile Tulip Tree in Western Kenya

Article *in* Plant Disease · January 2019

DOI: 10.1094/PDIS-07-18-1133-PDN

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M. Kyallo, Biosciences eastern and central Africa-International Livestock Research Institute (BecA-ILRI) Hub, P.O. Box 30709-00100, Nairobi, Kenya; **E. M. Ateka**, Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology, Nairobi P.O. Box 62000-00200, Kenya; **J. Ndunguru**, Mikocheni Agricultural Research Institute, Eastern zone, P.O. Box 6226, Dar es Salaam, Tanzania; **M. O. Ssemakula**, School of Agricultural Sciences, Makerere University, P.O. Box 7062, Kampala, Uganda; **R. A. Skilton**, International Centre of Insect Physiology and Ecology (ICIPE), P.O. Box 30772-00100, Nairobi, Kenya; **S. M. Kiarie**, Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology, Nairobi P.O. Box 62000-00200, Kenya; and **P. Sseruwagi**, Mikocheni Agricultural Research Institute, Eastern zone, P.O. Box 6226, Dar es Salaam, Tanzania.

Whitefly-transmitted cassava mosaic begomoviruses (CMBs; family *Geminiviridae*; genus *Begomovirus*) are viruses with bipartite genomes (components referred to as DNA-A and DNA-B) containing a conserved (between the two components) non-coding sequence known as the common region (CR) required for DNA replication and transcription (Fondong, 2013). CMBs cause cassava mosaic disease (CMD), a major limitation to cassava production in sub-Saharan Africa. In Kenya, CMD is associated with the begomovirus species *East African cassava mosaic virus* (EACMV), *African cassava mosaic virus* (ACMV), *East African cassava mosaic Kenya virus*, and *East African cassava mosaic Zanzibar virus* as well as several recombinant viruses, such as the virulent EACMV-Uganda (EACMV-UG) that so far occurs only in western Kenya (Nyongesa et al., 2016). Within the CMB-cassava pathosystem, various non-cassava plant species may play an epidemiological role as virus reservoirs for the whitefly vector *Bemisia tabaci* (Alabi et al., 2008).

In May 2017, leaf samples were collected from three plants exhibiting virus-like symptoms ranging from mosaic, mottling, twisted leaves, and reduction in leaf surface area as well as from two non-symptomatic plants belonging to the family *Bignoniaceae* growing in three cassava fields in western Kenya. Total genomic DNA was extracted from leaf samples using the ZR Plant/Seed DNA MiniPrep kit as per the manufacturer's instructions (Zymo Research Corp., USA). The identity of the plant species was confirmed as *Markhamia lutea* by phenotypic characteristics and PCR amplification, Sanger sequencing and BLASTn analysis of the chloroplast rubisco large-chain gene using the primer pair RbcLa-F (5'-ATGTCACCACAAACAGAGACTAAAGC-3')/ RbcLa-R (5'-GTAAAATCAAGTCCACCR CG-3'). Samples were screened by PCR with the diagnostic primer pairs JSP001/JSP002 for ACMV, JSP001/JSP003 for EACMV (Fondong et al., 2000), and UV AL1-F1/ACMV CP-R3 for EACMV-UG (Zhou et al., 1997). Samples from non-symptomatic plants did not yield amplification products, confirming absence of viral infection. A 1,650 bp fragment of the expected size was amplified from one symptomatic sample with the primer pair UV AL1-F1/ ACMV CP-R3, suggesting the presence of EACMV-UG. To confirm the positive PCR result, the genome of the virus was enriched by rolling-circle amplification (RCA) using a TempliPhi 100 RCA Kit (GE Healthcare, USA). The library was prepared using the NEBNext Ultra II DNA library prep kit for Illumina (New England Biolabs, USA) according to the manufacturer's instructions and sequenced using an Illumina MiSeq platform with the MiSeq Reagent Kit v3 (600 cycles) (Illumina, USA). Following *de-novo* sequence assembly, 344 and 89 paired-end reads aligned to generate two contigs identified by BLASTn search as the DNA-A and DNA-B of a bipartite virus. The

nucleotide sequences were deposited in GenBank as accession numbers MF974568 and MH379641. The 2,798 nucleotide (nt) DNA-A and 2,774 nt DNA-B have an approximately 160 nt EACMV-like CR with 53% nt sequence identity. DNA-A shares 99% nucleotide sequence identity with the DNA-A of a EACMV-UG isolate (AJ717522) from western Kenya, while the DNA-B is closely related to another EACMV-UG isolate (AJ704962) from western Kenya at 97% nucleotide sequence identity.

To the best of our knowledge, this is the first report of occurrence of EACMV-UG in a species of the family *Bignoniaceae*. Further investigations are required to determine the epidemiological significance of *M. lutea* in the CMB-cassava pathosystem.

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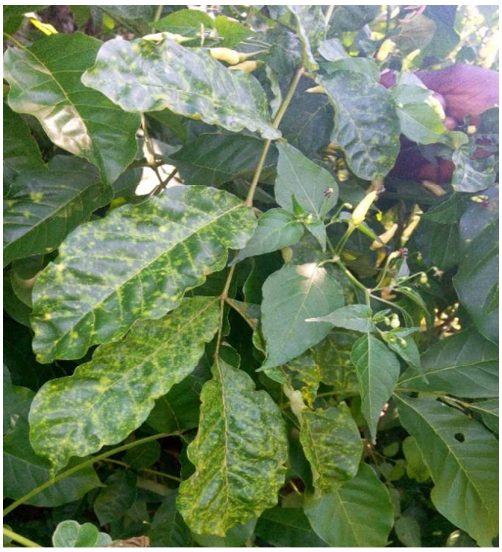

Supplementary Figure: Symptoms of yellow mosaic, mottling, and twisted leaf margins on leaves of Nile tulip (*Markhamia lutea*) tree infected with EACMV-UG.