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DISEASE NOTES

First Report of *East African cassava mosaic Malawi virus* in Plants Affected by Cassava Mosaic Disease in Zambia

R. M. Mulenga, Zambia Agriculture Research Institute, Mount Makulu Central Research Station, Chilanga, Lusaka Zambia; **D. W. Miano**, University of Nairobi, College of Agriculture and Veterinary Sciences, Department of Plant Sciences and Crop Protection, Kangemi, Nairobi, Kenya; **P. C. Chikoti**, Zambia Agriculture Research Institute, Mount Makulu Central Research Station, Chilanga, Lusaka Zambia; **J. Ndunguru**, Mikocheni Agricultural Research Institute, Dar es Salaam, Tanzania; **J. P. Legg**, International Institute of Tropical Agriculture, Dar es Salaam, Tanzania; and **O. J. Alabi**, Department of Plant Pathology & Microbiology, Texas A&M AgriLife Research and Extension Center, Weslaco, TX 78596.

ABSTRACT

Cassava mosaic disease (CMD) is a major constraint to sustainable production of cassava (*Manihot esculenta* Crantz) in Zambia and other sub-Saharan African countries. During a survey conducted between April and May 2014 in six (Western, Northwestern, Northern, Luapula, Lusaka, and Eastern) provinces of Zambia, 226 symptomatic cassava leaf tissue samples were collected from CMD-affected plants in 214 farmers' fields. PCR screening of these samples using species-specific primers targeting multiple cassava mosaic geminiviruses (Aloyce et al. 2013) revealed the presence of *African cassava mosaic virus*(ACMV), *East African cassava mosaic virus* (EACMV), and *East African cassava mosaic Malawi virus* (EACMMV) in the samples as single or mixed infections of different proportions. Considering that EACMMV is a previously unreported virus from Zambia, DNA extracts from three samples (ZM-LSK48 from Lusaka Province, ZM-E74 from Eastern province, and ZM-N112 from Northern province) showing severe symptoms of yellow mosaic and leaf distortion were subjected to rolling circle amplification (RCA, TempliPhi Amplification Kit, GE Healthcare Life Sciences, Uppsala, Sweden). The RCA product was shipped to Texas A&M AgriLife Research and Extension Center, Weslaco, TX, on FTA Classic Cards (Whatman International Ltd., Maidstone, UK) for further analysis under a permit from USDA-ARS-PPQ (P526P-14-04321). Using a pair of newly designed EACMMV-specific abutting primers (EACMMV-F: 5′-GTGATGTTACKCGTGGTTCG and EACMMV-R: 5′-ACCCTATGAGTAATACCCGA) and the RCA eluates as templates, the ≈3-Kb product obtained from each of the three samples was cloned into pCR2.1 vectors (Life Technologies, Carlsbad, CA, USA), and plasmid DNA from two independent clones per

sample was sequenced in both orientations. Additional virus-specific primers were utilized for primer-walking the plasmid DNA and resulting sequences assembled. A BLASTn analysis (Altschul et al. 1990) of the 2,804 nucleotide sequences derived from each of the two independent clones per sample (KP890349-354) revealed that they are specific to EACMMV. Neighbor-joining phylogenetic analysis (Tamura et al. 2013) with a diverse set of DNA-A sequences of cassava mosaic geminiviruses revealed the clustering of all six Zambian sequences within the EACMMV clade. Furthermore, the six sequences shared 98.5 to 99.9% identity among themselves, 98.1 to 98.5% with DNA-A of EACMMV isolate MK (AJ006460), and 98.3 to 98.8% with EACMMV isolate MH (AJ006459). Comparable levels of coat protein and replicase gene nucleotide and amino acid sequence identities were also obtained between EACMMV isolates from Zambia and corresponding sequences of global reference virus isolates. These results provide definitive evidence for the occurrence of EACMMV in Zambia and represent a first report of the virus in the country. It also indicates an expanded geographical distribution of EACMMV in sub-Saharan Africa.

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