DISTRIBUTION, MOLECULAR DETECTION AND CHARACTERIZATION OF CASSAVA MOSAIC GEMINIVIRUSES IN ZAMBIA

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DECLARATION

This is my original work and it has not been submitted for award of a degree in any other University

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ABSTRACT

Cassava mosaic disease (CMD) is a known constraint to cassava production in Africa. Its spread is driven primarily by three factors: planting of infected cuttings, use of susceptible varieties and viruliferous whitefly (*Bemisia tabaci*) populations. Although CMD-causing viruses produce characteristic foliar symptoms, a complex of viruses could be responsible. In Zambia, viruses infecting cassava have previously been reported as *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV). Specific virus species, their spread and nature of infection (whether single or mixed) have not been reported, nor have the viruses been characterized. Characterization and identification of viruses using sensitive, specific and robust diagnostic tools is an important step in the management of CMD. Therefore, the aim of this study was to conduct a virus survey in cassava fields in Zambia, determine their diversity and evaluate previously designed primers for detection of CMD-causing viruses in both uniplex and multiplex polymerase chain reaction (PCR).

A survey was conducted between April and May 2014 in 214 farmers' fields located across six major cassava-producing provinces of Zambia (Western, Northwestern, Northern, Luapula, Lusaka and Eastern) to determine the incidence and severity of CMD and the species diversity of associated cassava mosaic geminiviruses (CMGs). Mean CMD incidence varied across all six provinces but was greatest in Lusaka Province (81%) and least in Northern Province (44%). There was less variation in CMD severity between provinces, with mean scores ranging from 2.8 for Eastern Province to 3.0 for Northwestern Province. A total of 226 cassava leaf samples were collected and DNA extracted using Dellaporta method followed by sample analysis by PCR with degenerate and species specific primers. Over 91% of the 226 survey samples were positive in

PCR while 8.4% were negative. Of the positive samples, proportions of viruses in the samples were 62.8% ACMV, 5.8% *East African cassava mosaic Malawi virus (*EACMMV) and 2.2% EACMV, whilst mixed infections of ACMV+EACMV and ACMV+EACMMV occurred in 0.4% and 19.5% of samples, respectively. Mixtures of all the three ACMV+EACMV+EACMMV were found in 0.9% of the samples. Two primer pairs (ACMV1/2 and EACMMV1/2) that were previously used to detect cassava geminiviruses (CMGs) in Zambia were evaluated for their sensitivity and specificity to detect cassava mosaic geminiviruses in Zambian samples. Sensitivity was assessed using 10 to 10^{-5} diluted DNA extracted from fresh symptomatic cassava leaf samples whereas specificity was achieved by subjecting primers to samples that contained ACMV, EACMV and / or EACMMV. The detection limits for the two primers ACMV1/2 and EACMMV1/2 were optimum at dilution 10^{-2} (20-30 ng/µl) whereas DNA concentrations below or above this gave fewer detections. Collectively, the two primers detected viruses from 202 samples compared with 207 samples amplified with degenerate primers AV494/AC1048 in singleplex PCR. Of the 207 samples that were positive using the degenerate primer, 46 tested positive for both ACMV and EACMMV in duplex PCR. Analysis of the five samples that were not amplified with ACMV1/2 and EACMMV1/2 and were positive with AV494/AC1048 primers revealed that they contained EACMV. The discrimination of EACMMV from EACMV shows a high level of specificity of primer pair EACMMV1/2.

Since no sequences of viruses in Zambia were published prior to this study, 38 core coat protein (CCP) amplified PCR products were cloned and sequenced. Further, from the 38 samples, nine samples were subjected to rolling cycle amplification (RCA) and complete genomes amplified, cloned and sequenced. Analysis of resultant partial sequences revealed the presence of ACMV,

EACMV and EACMMV as single or mixed infections in differing proportions. This result was consistent with BLASTN results of complete DNA A genomes of 19 independent clones obtained from the nine samples. This study revealed the presence of previously unreported viruses in both single and mixed infections and further underscores the importance of the study in understanding the diversity of viruses causing CMD in Zambia and the need for studying the challenge they pose in the management of CMD in farmers' fields.