MOLECULAR CHARACTERIZATION OF CASSAVA MOSAIC GEMINIVIRUSES IN TANZANIA

by

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DEDICATION

This PhD thesis is a dedication to my Father Mr Canisius Manyahy Ndunguru and to the Lord for the gift of the Holy Spirit

DECLARATION LETTER

I, the undersigned, declare that the thesis, which I hereby submit for the degree of Doctor of Philosophy at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution

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Molecular characterization of cassava mosaic geminiviruses in Tanzania

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Abstract

Cassava (*Manihot esculenta* Crantz) is a basic staple food crop in Tanzania. Cassava mosaic disease (CMD) caused by cassava mosaic geminiviruses (CMGs) constitutes a major limiting factor to cassava production in the country. This study was undertaken to characterize the CMGs occurring in Tanzania using molecular techniques and to map their geographical distribution to generate information on which the formulation of control measures can be based. Using Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) for analysis of CMGs DNA-A genomes, different CMGs were found to be associated with CMD. Higher molecular diversity was observed among East African cassava mosaic viruses (EACMVs) than African cassava mosaic viruses (ACMVs), which was confirmed later by complete nucleotide sequence analysis. In addition to EACMV and ACMV isolates, two isolates of EACMV Cameroon virus (EACMCV) were found in Tanzania. These were confirmed to be strains of EACMCV Cameroon, originally described in Cameroon, West Africa and here named EACMCV- [TZ1] and EACMCV-[TZ7]. They had high (92%) overall DNA-A nucleotide sequence identity and EACMCV-[TZ1] was widespread in the southern part of the country. A

subgenomic DNA form of CMG that appeared to be truncated was identified in a CMD-infected cassava plant. It was confirmed upon sequence analysis to be a defect of EACMV DNA-A and had a capacity of attenuating symptoms when coinoculated with wild-type EACMV. In addition, this study revealed for the first time the presence of two novel non-geminivirus single-stranded DNA (ssDNA) sub-genomic molecules associated with CMG infection. They were shown to be dependent on CMG for replication and movement within the plants, confirming their status as satellite molecules named here as satDNA-II and satDNA-III. When present in coinfection with CMGs, they enhance symptoms and can break high levels of resistance in a cassava landrace. Finally a simple, inexpensive technique is described of archiving, transporting and recovering plant DNA for downstream geminivirus characterisation.

Key words: *African cassava mosaic virus*, cassava, cassava mosaic disease, cassava mosaic geminiviruses, *East African cassava mosaic virus*, DNA-A, DNA-B molecular characterisation, Tanzania

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CONTENT

LIST OF ACRONYMS AND ABBREVIATIONS

ACMV	African cassava mosaic virus
AYVV	Ageratum yellow vein virus
CBSD	cassava brown streak virus disease
CBSV	cassava brown streak virus
CMD	cassava mosaic disease
CMGs	cassava mosaic geminiviruses
CP	coat protein
CLV	cassava latent virus
CR	common region
CM	Cameroon
COSCA	collaborative study of cassava in Africa
ClcuMV	cotton leaf curl mosaic virus
^{o}C	degree Celsius
df	defective
DNA	deoxyribonucleic acid
DIFID	Department for International Development, UK
DI	defective interfering
DRC	Democratic Republic of Congo
EACMV	East African cassava mosaic virus
EACMMV	East African cassava mosaic Malawi virus
EACMZV	East African cassava mosaic Zanzibar virus
FAO	Food and Agricultural Organization of the United Nations
Fig.	figure
ICMV	Indian cassava mosaic virus
IITA	International Institute of Tropical Agriculture
ILTAB	International Laboratory for Tropical Agricultural Biotechnology
IR	intergenic region
kbp	kilobase pair
KE	Kenya
L	liters
MAFS	Ministry of Agriculture and Food Security

min	minutes
MAS	marker-assisted selection
MP	movement protein
NG	Nigeria
NSP	nuclear shuttle protein
ORF	open reading frame
PAUP	phylogenetic analysis using parsimony
PCR	polymerase chain reaction
PCNA	proliferating cell nuclear antigen
PNACL	protein and nucleic acid chemistry laboratory
REn	replication enhancer
RFLP	restriction fragment length polymorphism
satDNA	satellite deoxyribonucleic acid
SACMV	South African cassava mosaic virus
siRNA	short interfering ribonucleic acid
SLCMV	Sri-Lankan cassava mosaic virus
ssDNA	single-stranded deoxyribonucleic acid
SqLCV	squash leaf curl virus
TGMV	tomato golden mosaic virus
ToLCV	tomato leaf curl virus
TrAP	transcription activating protein
TZ	Tanzania
UG	Uganda
µl	microlitter
µg	microgram
WTGS	whitefly-transmitted geminiviruses

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