# SCREENING OF SELECTED CASSAVA CULTIVARS FOR SACMV RESISTANCE

**Rozida Haroon Osman** 

A research report submitted to the Faculty of Science, University of Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of Master of Science

Johannesburg, 2005

# DECLARATION

I declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Master of Science in Biotechnology in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

(Signature of canditate)

(Signature of Supervisor)

(Head of School)

(Head of Graduate Studies)

\_\_\_\_ day of March 2005

## ABSTRACT

Cassava is one of the most important staple crops in the world and is consumed by over 700 million people around the globe and is a profitable product commercially due to the high starch content of its tubers. One of the future aims is to produce cassava that is high yielding, resistant to cassava mosaic geminiviruses (CMGs) and high in starch content. To be able to achieve commercially attractive cassava varieties, research need to be carried out to investigate the virus resistance status of different cassava cultivars, which can later be used in the future breeding programme.

In South Africa, cassava is used for commercial starch manufacturing purposes, as a cash crop and a food source by small-scale farmers. Cassava Mosaic Disease (CMD) is having a negative impact on yield of the crop globally and therefore dropping profitability of cassava on a commercial scale.

The aims of this research were to propagate thirteen cassava cultivars and then to test them for virus susceptibility or resistance.

Eleven cassava cultivars received from the International Institute of Tropical Agriculture (IITA) were tested for resistance or susceptibility against South African cassava mosaic virus (SACMV). Two local, commercial cultivars T200 and T400, were tested for East African cassava mosaic virus (EACMV) and African cassava mosaic virus (ACMV) resistance.

Cassava cultivars were successfully propagated *in vitro* and thereafter transferred into soil and acclimatized to adapt to environmental conditions. When the plantlets were three weeks old, the plantlets were infected with cassava mosaic viruses. Plants were infected with SACMV via *Agrobacterium*-mediated transfer and infectious EACMV and ACMV monomers were used to biolistically bombard the plantlets.

Resistance/susceptibility results of seven of the thirteen cultivars were obtained with SACMV, these cultivars being T200 (susceptible), T400 (susceptible),

TME3 (highly resistant), I30572 (susceptible), I420251 (highly susceptible), I60506 (susceptible) and TMS60444 (susceptible). Due to destruction by fungal gnats eating the roots of the plants, acclimatization of the remaining six cultivars was not possible. Also, due to the nature of the biolistic equipment, infection of the cultivars with EACMV and ACMV was not achieved as the plantlets were not robust enough to survive the pressure.

Dedicated to : Parents, Sisters & Muhammed

# Acknowledgements

I would like to express my sincere gratitude to all those who assisted me in achieving my Masters degree - my supervisor Professor Chris Rey, co-supervisor Mr. Murunwa Makwarela, Sarah Taylor, Azola Fali and all my lab friends who provided me with endless support.

I would also like to thank the National Research Foundation for the grant they provided me with.

# **CONTENTS PAGE**

	PAGE
LIST OF FIGURES	XX
LIST OF TABLES	xxi
CHAPTER ONE – INTRODUCTION	
1.1 Overview	1 – 3
1.2 Cassava in South Africa	3 - 4
1.3 Constraints on Cassava Production	4-5
1.3.1 Major Pests	4 - 4
1.3.2 Major Diseases	4 – 5
1.4 Cassava Mosaic Disease (CMD)	5 - 9
1.4.1 Cassava Mosaic Disease in South Africa	7 - 9
1.5 Taxonomy of Cassava Mosaic Viruses	9 – 14
1.5.1 Begomovirus –Structure and Open Reading Frames	10 - 14
1.5.1.1 AV2 (Capsid Protein)	12 – 12
1.5.1.2 AL1 (Replication Protein)	12 – 12
1.5.1.3 AL2 (Transactivating Protein)	13 – 13
1.5.1.4 AL3 (Replication Enhancer Protein)	13 – 13
1.5.1.5 AL4	13 – 13
1.5.1.6 BR1 and BL1	13 – 13
1.5.1.7 Intergenic Region (IR)	13 – 14
1.6 The Viral Replication Cycle	14 – 15
1.7 Aims & Objectives	15 – 16

### CHAPTER TWO - ISOLATION & PURIFICATION OF DNA MONOMERS

2.1 Introduction	& Overview of Method	s 17 – 1	7

2.2 SACMV, EACMV & ACMV Infectious Clones	18 - 18
2.3 Materials & Methods	19 – 21
2.3.1 Precipitation of Cloned Vectors	19 – 19
2.3.2 Transformation into <i>E.coli</i>	19 – 20
2.3.3 Screening for Transformants	20 - 21
2.3.4 Restriction Enzyme Digests	21 – 21
2.4 <u>Results &amp; Discussion</u>	22 – 25
2.4.1 Precipitation of ACMV & EACMV Infectious Clones	22 - 22
2.4.2 Transformation	22 - 22
2.4.3 Screening for Transformants	22 - 23
2.4.4 Restriction Enzyme Digests	23 – 25

# CHAPTER THREE – *IN VITRO* PROPAGATION OF CASSAVA & ACCLIMATIZATION INTO GREENHOUSE CONDITIONS

3.1	Tissue Culture Overview	26 - 28
3.2	Materials & Methods	28 - 29
3.2.1	Establishment of in vitro cultures	28 - 28
3.2.2	Transferring into MS2-NAA-Phytogel	29 – 29
3.2.3	Growing Nicotiana benthamiana in vitro	29 – 29
3.2.4	Transfer into Soil & Acclimatizing	29 – 29
3.3	Results & Discussion	29 - 35

## CHAPTER FOUR – AGROINOCULATION & BIOLISTIC BOMBARDMENT

4.1	<b>Overview of Plant Transformation</b>	36 - 40
4.1.1	Agrobacterium Mediated Gene Transfer	36 - 38

4.1.2	Gene Transfer by Biolistic Bombardment	38 - 40
4.2	Materials & Methods	40 - 42
4.2.1	Preparation of Plantlets for Bombardment	40 - 40
4.2.2	Particle Preparation	41 – 41
4.2.3	Microprojectile Bombardment Procedure	41 – 41
4.2.4	Agroinoculation with SACMV	41 - 42
4.3	Results & Discussion	42 - 43

#### **CHAPTER FIVE – SCREENING FOR SYMPTOMS**

5.1	<b>Overview</b> - Screening for Resistance	44 – 46
5.2	Materials & Methods	46 – 46
5.2.1	Screening of Cassava Cultivars for Viral Infection	46 – 46
5.3	Results & Discussion	47 – 49

#### **CHAPTER SIX – CONCLUSIONS**

Conclusions	50 - 52

#### **APPENDICES**

REFERENCES

APPENDIX 1	57 - 63
1A – Ethanol Precipitation	57 – 57
1B – TSB Buffer	58 - 58
1C – Preparation of Luria Broth (LB) & LB Agar	58 - 58
1D – Plasmid Mini-Prep Solutions	58 - 58

53 - 56

1E – Preparation of 0.8% Gel	59 – 59
1F – 50% TAE Buffer	59 - 59
APPENDIX 2	60 - 60
2A – Preparation of MS2 Medium	60 - 60
2B – Preparation of MS2-Phytogel-NAA Medium	60 - 60
2C – Sterilization of N.Benthimiana Seeds	60 - 60
2D – Preparation of MS3 Medium	61 – 61
APPENDIX 3	62 - 62
3A – Preparation of Tungsten Particles	62 - 62
3B – Agro-inoculation	62 - 62
APPENDIX 4	63 - 63
4A – TNA Extraction	63 - 63
4B – PCR – Amplification of DNA	63 - 63

# **List of Figures**

## **CHAPTER ONE**

Figure 1.1	CMD in Different Regions of Southern Africa	7
Figure 1.2	Distribution of Cassava Mosaic Disease in South Africa	9
Figure 1.3	ORFs found on Geminiviruses	12

### **CHAPTER TWO**

Figure 2.1	Overview of Methods Used in Research	17
Figure 2.2	Map of TOPO Vector	18
Figure 2.3	Plasmid Mini-Prep of ACMV	23
Figure 2.4a-d	EcoR1 Restriction sites on ACMV & EACMV	24
Figure 2.5	Digested & Undigested ACMV-A and B	25

## **CHAPTER THREE**

Figure 3.1	Cassava Nodal Cuttings Transferred into	
	MS2-Phytogel-NAA Medium from MS2	31
Figure 3.2	Cassava Plantlets in MS2-Phytogel-NAA	
	Medium with Healthy Root & Shoot Formation	32
Figure 3.3	N.benthamiana in MS3 Medium Before	
	Transplantation	32
Figure 3.4a/b	Acclimatizing Plantlets Under High Humidity Conditions	34
Figure 3.5a/b	Acclimatized Cassava Plants from In vitro Conditions	34
Figure 3.6	Acclimatized N.benthamiana Plantlets	35

## **CHAPTER FIVE**

Figure 5.1a-d	Leaves of Infected	Cassava and <i>N.benthamiana</i> Plantlets	48
---------------	--------------------	--	----

# **List of Tables**

		PAGE				
CHAPTER ONE						
Table 1.1	Open Reading Frames on DNA A and DNA B of					
	Bipartite Begomoviruses	11				
CHAPTER TWO						
Table 2.1	Results of Transformation with ACMV & EACMV	22				
Table 2.2	EcoR1 Restriction Bands on EACMV/ACMV DNA-A					
	and DNA-B in TOPO Vector	25				
CHAPTER THREE						
TABLE 3.1	Number of Plantlets Acclimatized for Inoculation	30				
CHAPTER FOUR						
Table 4.1	Number of Cultivars Infected by Agro-inoculation &					
	Biolistic Inoculation	42				
CHAPTER FIVE						
Table 5.1	Scale for Screening CMD Symptoms	45				
Table 5.2	Resistance Status of Three South African Land Race					
	Cultivars and 10 Cassava Cultivars from IITA					
	Before This Study	46				
Table 5.3	Resistance Status of Cassava Cultivars to SACMV Two					
	weeks Post-inoculation	47				