Cassava mosaic and cassava brown streak diseases diagnostics: emerging capacities in African NARs

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Abstract

Cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) constitute the most formidable threat to cassava (*Manihot esculenta* Crantz) productivity in African continent. Effective management of CMD and CBSD depend very much on ability to diagnose them and their causal viruses efficiently, accurately and timely at low cost. In sub Saharan Africa capacity to diagnose CMD and CBSD is limited by deficiencies in array of interrelated factors. In 2008 we imitated a project aimed at enhancing the capacity of National Cassava Programs in Tanzania, Kenya, Uganda, Malawi, Rwanda, Mozambique and Zambia to develop diagnostics tools to effectively implement CMD and CBSD management programs. From 2008 to 2012, cassava virus disease diagnostic capacity in terms of human and infrastructure has been significantly enhanced in the project countries. NARs scientists have been trained on various cassava disease diagnostics. Various molecular laboratory disease diagnostic equipments were procured and delivered to NARs labs. As a result of these, cassava mosaic begomoviruses and cassava brown streak viruses as well as their associated vector, *Bemisia tabaci* have been identified and characterized using standardized and harmonized molecular tools. Cassava disease prevalent and virus distribution maps have been generated by NARs scientists to inform sustainable disease management strategies including provision of support to cassava seed systems and conventional breeding programs

Background

Cassava (*Manihot esculenta* Crantz) is an important root crop in many parts of the tropics, particularly in sub-Saharan Africa (SSA). Cassava mosaic disease (CMD), caused by *cassava mosaic begomoviruses* (CMBs) and cassava brown streak virus disease (CBSD) caused by *cassava brown streak viruses* (CBSVs) have seriously affected cassava productivity in Sub Saharan Africa. Sustainable management of these diseases is largely hampared by low capacities in diagnosis and monitoring of disease spread caused by deficiencies in an array of inter-related factors: training, soft and hard infrastructure, limited coordination and communication, baseline data, lab consumables and lack of effective, appropriate and comprehensive diagnostic tests as well as inadequate trained personnel.

Coordinated by Mickocheni Agricultural Research Institute (MARI) in Tanzania, a project was funded by the Bill & Melinda Gates Foundation in 2008 through 2012 to enhance the capacity of NARs in Tanzania, Kenya, Rwanda, Uganda, Malawi, Mozambique and Zambia to develop molecular diagnostic tools for sensitive and rapid detection of CMBs and CBSVs as well as conduct comprehensive cassava viruses identification, characterization, and develop disease prevalent maps in the project countries in order to effectively implement cassava disease management programs.

Table 1. Project implementing countries and institutions

Country	Institutions
Tanzania	Mikocheni Agricultural Research Institute (MARI)-*Leading and coordinating role*
Uganda	National Crops Resources, Research Institute (NaCCRI- NARO)
Kenya	Jomo Kenyatta University of Agriculture and Technology (JKUAT)
Rwanda	Rwanda Agriculture Board (RAB)
Mozambique	Agriculture Research Institute of Mozambique
Malawi	Department of Agriculture Research Services of Ministry of Agriculture and Food Security (MAFS)
Zambia	Zambia Agricultural Research Institute, Mt. Makulu Research Station

KEY PROJECT ACHIEVEMENTS

1. Enhancing infrustructure capacity

-National cassava virus disease diganostic capacity has been signifcantly enhanced in all the project countries

-Basic molecular disease diagnostic labs (PCR machines, gel documentation system and apparatus, centrifuges, fridges, GPS, Computers, water baths, pH meters) and lab consumables were procured and delivered to all the project countries

Figure 1. Lab facility in Rwanda

Figure 2. Procured project lab equipments at MARI in Mapudo, Mozambique. Similar equipments have been procured in the other project countries.

2) Develop robust standardized molecular diagnostic tools for sensitive and rapid detection of viruses infecting cassava

- Various PCR-based tools were developed and standardized for use by all project partners
- Comprehensive surveys completed in each of the project countries, samples collected were 286 (Rwanda), 259 (Tanzania), 280 (Zambia),410 (Kenya), 180 (Uganda) and 115 (Mozambique)
- Extraction of total DNA and RNA from collected leaf samples was completed using harmonized and standardized protocols

Figure 3.Sample collection in Rwanda

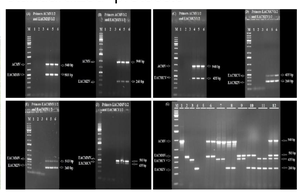

Figure 4. Single tube duplex and multiplex for simultenous detetction of CMBs

3. Conduct molecular characterization of the CMGs, CBSV and whitefly in the project countries

- Over 150 CBSVs coat protein gene sequenced
- 17 CMBs full DNA A genome s sequenced using 454 platform
- 486 whitefly indivudual collected from all the project countries were biotyped using MCO I gene primers and their phylogenetic relationships established

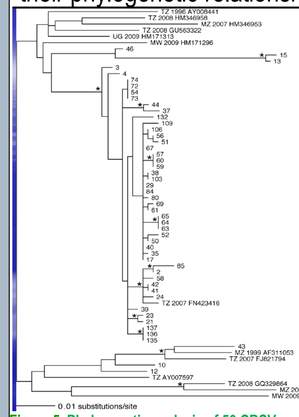

Figure 5. Phylogenetic analysis of 50 CBSV isolates from Tanzania compared with previous characterized isolates from the GenBank

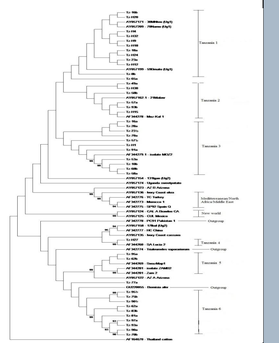

Figure 6. Phylogenetic tree of *Bemisia tabaci* biotypes from Tanzania denoted as Tz

4. Determine the status of cassava mosaic geminiviruses (CMGs) and CBSV in the project countries

- Status of *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV) established based on PCR-results.
- Disease prevalent maps for CMD and CBSD generated and now in use use other stakeholders involved in cassava improvement programs

Table 2. Status of CMBs in seven project countries by 2011

COUNTRY	ACMV	EACMV	A+E
Tanzania	8(3.1%)	134(51.9%)	6(2.3%)
Uganda	50(30.1%)	20(12)	20(12)
Kenya	29(8.0%)	176(49%)	
Rwanda	9(3.1%)	258(89.5%)	8(2.7)
Malawi	0	120(72.2%)	0
Zambia	59(30.8%)	39(20.4%)	15(7.8)
Mozambique	0	49(42.9%)	0
Total	155 (10%)	796(51.6%)	70(4.5%)

5. Enhancing human resource capacity in virus disease diagnostics

- over 20 NARS Scientists in cassava programmes from the project countries were trained on different cassava virus disease diagnostics: Field survey and sampling protocols; Data analysis and GIS mapping; DNA and RNA isolation techniques, PCR and RT-PCR analysis, primer designing and sequence analysis

Figure 7. Training of NARs scientists on disease survey, sample collection and laboratory analysis

6. To build capacity for cassava genetic transformation at MARI in Tanzania

- Capacity for cassava genetic transformation has been established at MARI in Tanzania after biosafety approval by the NBC
- Somatic embryos have been produced from 16 popular farmer-preferred CMD and CBSD-susceptible cassava cultivars
- First transformation was done using reporter gene construct
- 7 RNAi constructs made to confer resistance against CMBs and CBSVs MARI

Figure 8. Cassava somatic embryo produced from a landrace (Katakya) at MARI

Conclusion: Capacity of NARs to diagnose CMD and CBSD in SSA has been significantly enhanced. We do have a network of scientists working together using standardized diagnostic tools to address cassava disease problems, improve seed systems and provide practical solution to farmers while promoting biotechnology to improve agriculture in Africa.

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