The Cassava Diagnostics Project (CDP)

A review of 10 years of research

2008–2018

BILL & MELINDA GATES foundation

Regional Cassava Virus Disease Diagnostic Project

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Compiled by
Peter Sseruwagi, Fred Tairo and Joseph Ndunguru

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The Cassava Diagnostics Project (CDP): A review of 10 years of research 2008–2018

Peter Sseruwagi, Fred Tairo and Joseph Ndunguru

Tanzania Agricultural Research Institute (TARI)–Mikocheni
P.O. Box 6226
Dar es Salaam
Tanzania
Fax: +255 222775549
Phone: +255 222700552
E-mail: cmmikocheni@tari.go.tz

AGROVOC descriptors in English: 1. Manihot esculenta. 2. Cassava brown streak disease. 3. Cassava brown streak viruses. 4. Potyviruses, 5. Symptoms enhancing geminivirus symptoms (SEGS). 5. Non-cassava host plants. 7. Sub-Saharan Africa (SSA). 8. Outreach. 9. Support to breeders. 10. Disease diagnostics. 11. Pest insects. 12. Aleyrodidae. 13. Bemisia tabaci. 14. Cassava mosaic virus. 15. Geminiviruses. 16. Vectors. 17. Food security. 18. Poverty. 19. Pest control. 20. Disease control. 21. Integrated control. 22. Socioeconomic environment. 23. PCR. 24. Mixed cropping. 25. Highlands. 26. Capacity building. 27. Africa.

Local descriptors in English:
1. Cassava. 2. Whitefly. 3. Molecular techniques.
AGRIS subject categories:
H10 Pests of plants
H20 Plant diseases

ISBN 978-9976-59-336-5

Editorial, data analysis and data visualization support
provided by Scriptoria through AgShare.Today (http://agshare.today)

Suggested citation for this publication: Sseruwagi, P., Tairo, F. and Ndunguru, J. (2018) *The Cassava Diagnostics Project (CDP): A review of 10 years of research 2008–2018*. Tanzania Agricultural Research Institute (TARI)–Mikocheni: Dar es Salaam, Tanzania.

Suggested citation for a chapter: Tairo et al. (2018) 'Tanzania' In *The Cassava Diagnostics Project (CDP): A review of 10 years of research 2008–2018*. Sseruwagi, P., Tairo, F. and Ndunguru, J. (Eds). Tanzania Agricultural Research Institute (TARI)–Mikocheni: Dar es Salaam, Tanzania. pp 15–79.

DEDICATION

We dedicate this Monograph to the memory of Shubi Katagira, who faithfully served as the project accountant at TARI-Mikocheni for many years.

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Bill & Melinda Gates Foundation (BMGF)

The Bill & Melinda Gates Foundation (BMGF) is a private organization founded by Bill and Melinda Gates that aims to enhance healthcare and reduce extreme poverty across the globe. One of BMGF's areas of focus is agricultural development based around the key principles of fostering breakthrough discoveries in scientific research, strengthening the tools available to farmers across the world, and providing better data systems for agriculture.

UK Department for International Development (DFID)

The Department for International Development (DFID) is a United Kingdom government department responsible for administering overseas aid. The goal of the department is "to promote sustainable development and eliminate world poverty". It supports research and programs around the globe aimed at ending extreme poverty.

ABOUT THE PARTNERS

Tanzania Agricultural Research Institute (TARI)–Mikocheni, Tanzania

The Tanzania Agricultural Research Institute (TARI)–Mikocheni is a public agricultural research institute under the Ministry of Agriculture in Tanzania. Among its mandates is conducting and promoting agricultural biotechnology research for socio-economic development in the country.

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COUNTRIES TARGETED FOR CDP IMPLEMENTATION

INTRODUCTION

Joseph Ndunguru
Project Coordinator, Cassava Diagnostics Project

Peter Sseruwagi and Fred Tairo
Assistant Project Coordinators, Cassava Diagnostics Project

Tanzania Agricultural Research Institute (TARI)–Mikocheni

Cassava virus research and sustainable disease management in East and Southern Africa

About 60% of the world's arable land is in Africa (FARA, 2014). The overall African population engaged in agriculture is estimated at 530 million and is expected to exceed 580 million by 2020 (NEPAD, 2013). The proportion of the population in Africa reliant on agriculture is 48% and in East Africa this figure is almost 70%. Agriculture is the predominant source of employment and livelihood and a way of life for most African citizens. This sector provides employment for 50% of the labor force – 47% of these workers are women and the majority of farmers are subsistence farmers. However, the agricultural sector provides only an average of 15% of GDP to the continent (ADB, 2016). The increasingly low contribution of agriculture to the economy is a sign of low productivity and limited value addition.

The Science Agenda for Agriculture in Africa (FARA, 2014) is an African-owned and African-led process that emphasizes the application of science and technology for agriculture to double agricultural productivity by 2025 and connect science to the end-users. Africa has the potential to increase the value of its annual output from about $280 billion (in the late 2000s) to around $800 billion by 2030 (McKinsey Global Institute, MGI 2010). There is a consensus within Africa that such a vital sector as agriculture needs to be transformed using the catalytic power of science and technology.

In this monograph, using cassava (*Manihot esculenta* Crantz) as a model crop, we demonstrate how the application of science and technology can play a key role in transforming agriculture in Africa and how this can benefit smallholder farmers.

Cassava is an important source of food in developing countries after maize, rice and wheat and is grown mainly by subsistence and small-scale farmers. This root crop is a source food for an estimated 800 million people in Asia and in the tropics – about 300 million people in sub-Saharan Africa (SSA) depend on cassava as their source of daily calories. It is a staple food throughout Africa – that is, an item of good nutritional value that is consumed as the major part of the daily diet of a large part of a country's population – as is the case in the seven countries that participated in the Cassava Diagnostics Project (CDP).

Cassava is well adapted to poor soils, and its economically desirable attributes include drought tolerance and the ability to store the roots for lengthy periods in the ground, allowing flexible

harvesting over long periods. Thus, cassava is considered a major food security crop in many developing countries. Additionally, cassava is a valuable source of starch with potential industrial uses, such as in animal feed, biofuel, paper, textiles and food processing applications. Overall, healthy cassava can contribute nutritionally and economically to the countries where it is grown.

However, the benefits of this crop are seldom felt in the East and Southern African countries where cassava is grown. Among the many challenges farmers face are plant diseases that significantly reduce crop yields, often caused by viral pathogens. Viral diseases are difficult to prevent, and once established there are few ways to counter their impact on yield. As a result, development and deployment of resistant crop varieties remains the most effective manner to combat the evolving threats of plant viral diseases. Underpinning such efforts is the need for robust diagnostic capacities to identify the species and strains of viral pathogens infecting crop plants and their associated vectors, as well as their related wild species, and to understand their distribution within a given geographical region.

To achieve this aim, in-depth studies of the pathogens using state-of-the-art molecular tools can detect evolutionary or environmental changes and inform the management of viral diseases so as to maximize crop yields for farmers. Another important factor in combating plant diseases is to understand the means by which crops become infected and how the diseases are spread at local level – from farm to farm, village to village – and further afield to district and regional levels.

An integrated approach is required to translate the knowledge gained in the laboratory into solutions that can be incorporated into the farmers' practices so that they adopt them readily. Diagnostic capacities must be simple, cost-effective and robust enough to be used in resource-constrained countries like those in SSA.

In order to achieve this aim, a formal phytosanitary framework is required to (1) develop disease-free cassava plants by plant breeders, (2) make disease-free planting materials available to farmers, (3) formulate an education program so that the farmers recognize cassava viral disease symptoms on their crops and (4) train extension workers involved in outreach programs so that they can provide assistance to farmers. To enable the project to meet these aims, funding was received from the Bill & Melinda Gates Foundation (BMGF) and the UK Department for International Development.

Cassava viral diseases

The main viral pathogens constraining healthy cassava production in East and Southern Africa are cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). CMD is widespread in SSA, whereas CBSD has been reported from East African countries and around the Great Lakes region.

Both CMD and CBSD have seriously reduced yields across the continent, often forcing farmers to abandon their fields. In 2005 for example, CMD alone caused crop losses of 4 million t/year in Tanzania, Uganda, Rwanda and Burundi. It is estimated that US$2–3 billion is lost annually due to CMD in SSA (Scholthof et al., 2011). These viral diseases have caused severe food shortages among resource-poor farmers in Africa, threatening food security across the continent.

Symptoms of CMD in cassava vary from mild mosaic to severe symptoms of leaf curl, leaf distortion, yellowing and plant stunting – depending on the infected cultivar, virus species/strain (mixed infections are common) and climatic conditions. The major symptom types in CBSD include yellowing

of older leaves along the major veins, round or elongated brown streak-like lesions on the stems and, most destructively, production of brown, corky, necrotic lesions within the storage roots.

Root and leaf symptoms of CBSD are highly variable and depend on host genotype, virus species and environmental conditions. Co-infection with more than one virus species or strain is a common feature in the etiology of CBSD, often leading to increased symptom severity.

Cassava mosaic disease is caused by cassava mosaic begomoviruses (CMBs) (family *Geminiviridae*; genus *Begomovirus*). The virus is spread by the whitefly *Bemisia tabaci* Gennadius (Hemiptera) (Dubern, 1994) and also through movement of CMB-infected planting material (Harrison, 1987). In SSA, there are eight recorded cassava-infecting CMBs (Brown et al., 2015) including: *African cassava mosaic virus* (ACMV), *Cassava mosaic Madagascar virus* (CMMGV), *East African cassava mosaic virus* (EACMV), *East African cassava mosaic Cameroon virus* (EACMCV), *East African cassava mosaic Malawi virus* (EACMMV), *East African cassava mosaic Kenya virus* (EACMKV), *East African cassava mosaic Zanzibar virus* (EACMZV) and *South African cassava mosaic virus* (SACMV).

Early studies on the distribution of CMBs in SSA indicated that ACMV was widespread in most cassava-growing areas in west, central, eastern and southern Africa, with the exception of the coastal areas of Kenya, Tanzania and Mozambique. Indeed, ACMV is considered one of the top 10 pathogens affecting cassava.

In contrast, EACMV was believed to be restricted to coastal Kenya, Tanzania, Mozambique, Zanzibar and Madagascar, and some areas of Malawi (Harrison et al., 1991, 1996). However, subsequent studies showed that EACMV also occurred inland in western Kenya and western Tanzania (Gibson et al., 1996; Legg et al., 1999; Were et al., 2003, 2004). In West Africa (Cameroon, Guinea, Ivory Coast, Nigeria, Senegal and Togo), EACMCV is the predominant species (Fondong et al., 2000; Ogbe et al., 2003, 2006; Okao-Okuja et al., 2004).

These viruses are transmitted by the polyphagous cryptic whitefly complex *B. tabaci*, mechanically transmitted through grafting and sap inoculation of herbaceous plant species, and are propagated through infected stem cuttings.

Whitefly

About the organism

Whiteflies *Bemisia tabaci* (Gennadius) (*Hemiptera: Aleyrodidae*) are phloem (sap) feeders. They cause direct damage in some hosts by extracting large quantities of sap. *Bemisia tabaci* is a vector of plant begomoviruses of the *Geminiviridae*. These whitefly-transmitted viruses (WTV) are among the most destructive plant viruses and early virus infection can result in total crop loss.

Although the problems caused by *B. tabaci*, both as pest and vector, have been recognized for more than 100 years, serious damage had been limited to a handful of crops in particular geographic areas. This scenario has changed over the past two decades. The known WTVs have extended their geographic range and other WTVs are emerging in new crops and geographic zones around the world. Whitefly infestations have become severe in both traditional and non-traditional food and industrial crops throughout the tropics.

Role in CMD and CBSD

A key feature of the geographical areas severely affected by CMD and CBSD is the presence of high whitefly populations on cassava plants. The persistent mechanism of transmission of CMBs by *B. tabaci* (viruses can be retained up to 9 days) facilitates long-distance movement of virus populations (up to 38 km in a year) and has important consequences for the pattern of virus spread. Examples of the steady progression of these diseases can be found in published literature (for example, Otim-Nape et al., 1997, 2000; Legg, 1999; Pita et al., 2001). These studies demonstrate changes in the viruses as well as documenting their route from northern Uganda moving southwards at approximately 20 km/year and reaching Kenya. In both countries, severe to total crop failure was experienced.

With these historical events in mind, one aim of the CDP project was to gain a better understanding of the whitefly and other insects that might contribute to the viruses causing CMD and CBSD. To this end, the collection of whitefly and other insects found on the plants became part of the surveys for plant material sampling to assess these insects. Although not all insects collected were studied comprehensively, they were identified to group or family level. The findings of the whitefly and insect assessments are detailed under the individual country chapters. The work carried out by individual partners also shows that whitefly may not be solely responsible for the spread of the viruses causing CMD and CBSD and that the dissemination of infected plant cuttings may play an important role in this problem.

Making disease-free cassava plants available to farmers

This has been a major objective for all the partner countries. Efforts to achieve this objective are described in the individual chapters of this document. It is widely acknowledged that, in addition to interacting with plant breeders, it is essential that farmers trust the material they are acquiring from the breeders. A formal certification system is thus vital to the success of the endeavor to manage CMD and CBSD. Tanzania has in place the Tanzania Official Seed Certification Institute.

Interaction with agricultural extension workers and farmers

From the start of the project in all partner countries, trust was built between project teams, government agricultural extension departments, local government administration and farmers. Their permission was sought in surveying their fields, and it was made clear to them that their cooperation was valued and essential to combat the disease problems they face. Farmers whose land was surveyed were taught how to recognize plant disease, and plots were designed and cultivated with their preferred cassava varieties as well as disease-free varieties in order to demonstrate the benefits of using clean planting material. Agricultural shows, printed material, radio and television broadcasts and other educational events were used to inform farmers about accessing clean planting material. These endeavors are described in detail within the individual chapters of this document.

In addition to direct interaction with farmers, CDP worked closely with extension staff. These staff can usually communicate with farmers in their dialect and offer practical assistance. As with farmers, it is essential that extension staff can recognize cassava diseases. Project teams were therefore active in providing training and information to this sector of the workforce – their work is detailed within the individual chapters of this document.

Disease diagnostic protocols

The overall aim of the project was to manage cassava virus diseases and their associated vectors sustainably using standardized and harmonized protocols. Among the protocols developed by CDP and shared among the network countries and beyond included that for disease surveillance (Sseruwagi et al., 2017). The parameters standardized and harmonized included a disease severity scoring scale (1–5), assessment of CMD and CBSD incidences, sample collection and determination of whitefly abundances in the cassava fields. The second protocol was the establishment of cassava demonstration plots (Sseruwagi et al., 2014), with standards developed for their design and size. Finally, laboratory disease diagnostic protocols were developed, standardized and harmonized, including sample storage, processing, primer design, PCR and results analysis. These protocols were shared through training, joint surveys and technical backstopping visits by project scientists. All CDP countries used the same protocols in their respective countries. In addition, a number of PCR primers for detection of various *cassava brown streak virus* and CMB species/strains were developed and shared widely for routine use in indexing cassava planting materials before materials were provided to farmers. All the protocols listed above were also shared with scientists in the WAVE (West African Virus Epidemiology) project for use in West African countries.

Origin and organization of the CDP

The goal of the CDP was formally stated and discussed at the Project Inception Meeting in December 2008. Using laboratory-based evidence that the capacity to diagnose disease is essential for effective and sustainable control of CMD and CBSD, the project aimed to enhance capacity to diagnose, characterize, monitor and sustainably manage viruses affecting cassava productivity. Partners within the CDP project were Tanzania, Kenya, Uganda, Rwanda, Mozambique, Malawi and Zambia. The general goal of the project was to improve cassava production in the region. Key gaps were highlighted by the partners. All partner countries lacked reliable information concerning cassava disease problems such as correct identification of diseases and hotspots, baseline data, surveillance, disease mapping for distribution, and diagnostics linked to resistance breeding. These criteria were considered vital to assist the National Agricultural Research Systems (NARS) in predicting disease spread and formulating control options.

In order to achieve this goal, CDP developed an effective diagnostic tool – that was simple, cost-effective and robust – to enhance the capacity of NARS in modern molecular diagnostics. An overview of the project budget and the disbursement procedures to participating countries was presented.

The key budget lines explained were Personnel and Administration – each partner country was headed by one scientist and a research assistant covered by the country's budget, but it was stressed that as all partners were government employees at their respective NARS, no fringe benefits would be issued because this would be the contribution of each country's government to the project. Regional and local travel would follow the partner country's government rates.

Equipment allocation guidelines were such that all countries had the same funds for similar types of equipment. The equipment was either purchased centrally by the Principal Investigator (PI) and shipped to respective partners, or each country purchased the material themselves depending on their procurement procedures.

A small budget was set for contract services, mainly the sequencing of isolates from partner countries by other advanced countries with sequencing facilities, where these are not present in partner countries.

There were no sub-grants or consultancies as there was no budget for such expenditure, but for all participating countries, 10% of direct costs could be charged by the institution to support administrative issues rendered to the project.

Provision was made for four MSc students, from CDP partner countries, to be supported by the project starting in year two. Partner scientists attending annual rotational workshops and meetings were also supported financially by the project. The organizing countries were supported by the PI office in using the allocated budgets. Funds were disbursed annually following proper accounting of previous disbursement at the agreed reporting date as per the project contract.

Common challenges for all partners included lack of information sharing, lack of staff training and retention of key project staff rather than them leaving for other employment with attractive packages within the region/partner country. Partners were reassured that attractive top-ups equivalent to other programs within the region would be provided to country team leaders to support them.

The procurement of key equipment and consumables was addressed because some partner countries had difficult procedures. A solution was to have central procurement points where the supplier could be directed by the PI office to deliver the consignment to the respective countries – this would take into account special cases where countries might require formal supporting letters for equipment provided for researchers by a donor.

Impact

The CDP had a significant impact both direct and indirect in all seven project network countries and beyond following its implementation. Over the ten years (2008–2018) that the CDP team worked together they sustained the network and performed its research goal of addressing cassava problems using harmonized strategies. Some of the key visible results of CDP are described below.

Both the human and infrastructure disease diagnostic capacity in the project countries was strengthened through training and establishment of laboratory facilities. Researchers in the project countries can now go to the field, collect disease and pest samples for analysis in the laboratories, and use the laboratory results to inform pest and disease management strategies.

University students and other research scientists in the project countries who might have left and gone abroad to conduct research work are now using the laboratory and greenhouse infrastructure established by the CDP project to conduct their research – a noticeable benefit for the project and their country.

Organizations such as the International Institute of Tropical Agriculture, research institutes, local government, government departments and NGOs involved in cassava improvement in the project countries are using information generated by the project. A good example is Tanzania in which local government in all the major cassava-growing areas received and used disease prevalence maps

generated by CDP. These maps contributed to decisions on where to multiply, screen and deploy cassava varieties, as well as strengthen phytosanitary regulations.

The visibility of the research institutes hosting the CDP increased, and resulted in these institutions attracting more funding through new collaborative projects and grants. These new collaborations have led to joint projects, publications and exchange visits, as well as the acquisition of new laboratory infrastructure. The CDP inspired many young research scientists in Africa to write grant-winning proposals for initiatives such as the Program for Emerging Agricultural Research Leaders (PEARL) funded by the Bill & Melinda Gates Foundation.

For smallholders within the CDP partner countries, the impact of the project has been positive. Those farmers who received virus-indexed clean planting cassava material through the project recorded yield increases from 5 to up to 40 t/ha. This has resulted in increased income and household food security.

Further afield, CDP supported the initiation of the WAVE project which addresses similar cassava virus diseases and their associated vectors in West Africa using a CDP model.

Challenges

From a science point of view, although *B. tabaci* was found to transmit CBSD, the transmission percentage was low and thus involvement of other agents in CBSD transmission cannot be definitively excluded.

Running regional projects such as the CDP include dealing with the human, administrative and logistical elements. The diversity of people, countries, government procedures, institutional arrangements and capacities influence project implementation. Practical challenges include slow procurement processes due to long tendering procedures and expensive laboratory equipment and supplies – often due to involvement of brokers as well as burdensome customs regulations and laws in some countries causing delays in delivery of laboratory supplies. Furthermore, biosafety regulations are not in place in some countries and there is low level of awareness among the public on application of biotechnology for crop improvement.

Conclusions

A number of stakeholders benefited from the implementation of the CDP – there is now a strong connection with the end-users of the science and technology. Farmers who used to get less than 5 t/ha of cassava before the project intervention are now getting 20–45 t/ha after receiving virus-free improved cassava material from the project. This has significantly contributed to improved household food security and income. The CDP also contributed significantly to enhanced human and infrastructure in the project countries through the acquisition of laboratory equipment and screenhouses and training of young scientists. This, in turn, contributed to the emerging of bioscience capacity in Africa – leading to the realization of the vision of seeing bioscience playing a key role in agricultural transformation in Africa.

The success of the CDP greatly depended on teamwork and notable attributes displayed by the team. These include: dedication and commitment, self-motivation, a high level of cooperation

among the project teams, and a high degree of transparency especially from the project leadership. Additionally, effective communication, consistent hard work and a high level of government support in all the project countries was instrumental.

Recommendations for future work

The Cassava Diagnostics Project is a good model to use for other staple crops. Researchers could benefit from our experience on a number of areas: How to start a large project? How best to find partners for collaboration? Could calls for proposals be a useful source for finding potential partners or seeing who else is addressing similar areas of research?

The project has demonstrated the value of cooperation between plant pathologists and plant breeders in a range of areas such as:

- Having the scientific capability to investigate complex scientific as well as practical problems and to provide training programs
- Advising on, or assisting with, setting up the required infrastructure for breeders to carry out their work efficiently
- Understanding the challenges faced by breeders and proposing solutions that fit readily with their usual operations
- Providing information to government bodies to highlight the potential benefits of this cooperation
- Fostering trust and respect in all areas of the project – scientific, technical and administrative. This is of paramount importance among partners in a project such as CDP
- Fostering trust and respect between scientists and farmers, i.e. treating farmers as partners in the endeavor
- Taking responsibility to share knowledge – scientific, technical as well as administrative know-how
- Using a common platform to which all partners have access – so that information is easily available
- Practicing a philosophy of cooperation: *If you wish to receive, you must be prepared to give*.

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TANZANIA

Fred Tairo, Peter Sseruwagi, Charles Kayuki, Hilda Bachwenkizi, Tarcisuis Fute, Deogratius Mark, Ramadhan Lipala, Laurencia Mushi, Joel Erasto, Shamsa Kileo, Rahma Mkangwa, Veneranda Mlegi, Margareth Lupembe, Veneranda Ngazi, Maliha Saggaf, Christina Kidulile, Dickson Lwabulala, Cyprian Rajabu and Joseph Ndunguru

Tanzania Agricultural Research Institute (TARI)–Mikocheni
P.O. Box 6226, Dar es Salaam, Tanzania

Abstract

For the past ten years, from 2008 to 2018, the Tanzania Agricultural Research Institute (TARI)–Mikocheni, in collaboration with six East and South African countries, has executed a regional project to enhance the diagnostic capacity and management of cassava virus diseases: cassava mosaic disease and cassava brown streak disease. The work carried out in Tanzania was part of the Cassava Diagnostics Project (CDP), and was executed in two phases: Phase I from 2008 to 2012 and Phase II from 2013 to 2018.

The CDP approach entailed addressing three major aims. The first aim was to understand the threat of emerging viruses and vectors. To achieve this, TARI–Mikocheni carried out four country-wide surveys. These surveys made it possible firstly to determine the incidence, severity and abundance of whitefly in 16 regions; secondly to determine the identity and diversity of 28 prevalent cassava brown streak viruses and Ugandan cassava brown streak viruses; thirdly to determine the identity and diversity of 13 cassava mosaic begomoviruses; and lastly to determine the epidemiology of CBSD and its spread within cassava fields.

Our second aim was to support seed systems. To achieve this, we contributed to the establishment of a fully functioning seed system for cassava by delivering more than 870,000 virus-tested cassava planting materials to over 500 cassava farmers.

To achieve the third aim of capacity-building, CDP enhanced the human and infrastructure capacity of TARI–Mikocheni. To this end, virus management training was provided to 14 postgraduate students and 48 senior scientists and research assistants. The infrastructure was improved through the refurbishment of three screenhouses and installing modern diagnostic equipment in three laboratories.

The work carried out by the CDP team in Tanzania and its results and impact are discussed in this chapter. Additionally, because TARI–Mikocheni was the hub for CDP, this chapter also addresses briefly aspects of work carried out at TARI–Mikocheni to assist our partners – these specific work items are dealt with in detail in the appropriate country chapters.

Acronyms and abbreviations

ACMV	*African cassava mosaic virus*
BecA-ILRI	Biosciences eastern and central Africa-International Livestock Research Institute
5CP	Cassava Varieties and Clean Seed to Combat CBSD and CMD project
CBSD	Cassava brown streak disease
CBSV	*Cassava brown streak virus*
CDP	Cassava Diagnostics Project
CMB	Cassava mosaic begomovirus
CMD	Cassava mosaic disease
COSTECH	Commission of Science and Technology
CP	Coat protein
DSMZ	Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH
EACMV	*East African cassava mosaic Cameroon virus*
FAO	Food and Agriculture Organization of the United Nations
GMO	Genetically modified organism
ICGEB	International Center for Genetic Engineering and Biotechnology
ICRAF	International Centre for Research in Agroforestry
IgG	Immuno globulin
JKUAT	Jomo Kenyatta University of Agriculture and Technology, Kenya
LAMP	Loop mediated isothermal amplification
MAI	Months after inoculation
MAK-UG	Makerere University of Kampala, Uganda
MAP	Months after planting
MEDA	Mennonite Economic Development Associates
NARS	National cassava research programs, Tanzania
NCSU	North Carolina State University, USA

NGS	Next-generation sequencing
PHS	Plant health services
PolyTech.KE	Kenya Polytechnic College
QTL	Quantitative trait locus
SEGS	Sequences enhancing geminivirus infection
SRI	Sugarcane Research Institute
SSA1-SG1	Sub Saharan Africa 1 sub genomic group 1 whitefly species under *Bemisia tabaci*
SUA-TZ	Sokoine University of Agriculture, Tanzania
TARI	Tanzania Agricultural Research Institute
TOSCI	Tanzania Official Seed Certification Institute
UCBSV	*Uganda cassava brown streak virus*
UoN-KE	University of Nairobi, Kenya
USAID	United States Agency for International Development
UMA-SP	University of Málaga – Spain
UWA	University of Western Australia
VIGS	Virus-induced gene silencing
Wits	University of the Witwatersrand, South Africa
ZARI	Zambia Agriculture Research Institute

Results summary: Tanzania

Aim I: Understand the threat from evolving viruses and vectors

Objective 1: Disease epidemiology

Disease and whitefly prevalence surveys conducted	• Four country-wide surveys were conducted in 2009, 2013, 2015 and 2017 in 786 cassava fields in 16 regions. • Of the 786 fields surveyed in four surveys, 687 were cassava mosaic disease (CMD) and 488 were cassava brown streak disease (CBSD) infected, respectively. • Mean CMD incidence (%) and symptom severity scale were: 44.36 & 3.36, 29.6 & 3.51, 28.84 & 3.36, and 25.53 & 3.0 in 2009, 2013, 2015 and 2017, respectively. • Mean cassava brown streak disease (CBSD) incidence (%) and symptom severity scale were: 42.20 & 3.0, 32.6 & 2.21, 36.5 & 2.43 and 18.71 & 2.53 in 2009, 2013, 2015 and 2017, respectively. • Mean whitefly populations/field during survey years were 7.35, 2.59, 7.75 and 8.31 in 2009, 2013, 2015 and 2017, respectively. • In 2016, TARI–Mikocheni, in collaboration with the modeling group at Cambridge and Rothamsted of the UK, cleaned and validated survey data and transferred them from hard copy into new Excel data sheets developed. These were for use on a tablet so that the data could be uploaded to the AgShare.Today platform. • A new standard operating procedure (SOP) for 2017 countywide survey developed and used to collect 2017 data.
Spread of CBSD within and between cassava fields	• Research on spatial and temporal spread of CBSD within cassava fields completed in 2014 reported in Gwandu et al. (2015) and MSc thesis (Gwandu, 2015, unpublished).
Alternative hosts for CBSVs and CMBs and associated insect vectors identified	• One *East African cassava mosaic virus* (EACMV)-like whole genome sequence obtained for a non-cassava shrub in Tanzania and published in Kyallo et al. (2017).
Nature of interaction between CBSVs and CMBs/SEGS and its impact on development of disease determined	• PhD student Cyprian Rajabu relocated from the University of the Witwatersrand, South Africa (Wits, SA) to North Carolina State University (NCSU), USA. • Two infectious clones for *African cassava mosaic virus* (ACMV)-CM DNA A&B components and SEGS1 cloned into pUC119 and transformed into DαH cells. • Graft inoculation of Arabidopsis with ACMV, SEGS1 and *Cabbage leaf curly virus* achieved. • Data on CBSVs/SEGS reported in Maliha (2018) MSc thesis, unpublished. • Data on CMBs interaction with SEGS reported in Rajabu (2018) PhD thesis, unpublished.

Objective 2: Characterization of emerging viruses	
Cassava virus isolates in the project countries sequenced and analyzed	• Cassava mosaic begomovirus isolates: 16 whole-genome sequences were obtained for CMBs in Uganda, Kenya, Malawi, Mozambique, Rwanda, Tanzania and Zambia. One manuscript to report the viruses under development (Sseruwagi et al., unpublished) is in advanced stages and was due for submission to a journal by end of March 2016. • Three CBSV isolates and three *Ugandan cassava brown streak virus* (UCBSV) isolates were obtained from Tanzania. The samples were deep sequenced at BecA-ILRI, Nairobi and bioinformatics analyses were done at the University of Western Australia (UWA) in November–December 2014; outputs are published in Ndunguru et al. (2015). • Additional 16 CBSV isolates were obtained on 2016 from Tanzania, sequenced at Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH (DSMZ) in Germany and a paper on 'Variability in P1 gene redefines phylogenetic relationships among cassava brown streak viruses' was jointly published in collaboration with Malawi isolates (Mbewe et al., 2017). • Additional 13 CMB isolates from cassava relatives were collected from non-cassava producing region in 2016, sequenced and published in Tairo et al., (2017).
Cassava virus distribution maps for partner countries, generated (incidence, severity, whitefly, viruses, sat)	• Maps produced by the individual partners were submitted to TARI–Mikocheni for compilation. • GIS expert engaged to develop standardized maps for all the project countries. • 300 disease maps generated from 2009, 2013 and updated with 2015 data, were shared with key stakeholders in the country. New disease maps were generated by Agshare.Today using survey data collected in 2017 and shared with stakeholders. Updated disease maps were made and will be published in a joint paper led by TARI–Mikocheni. • Disease maps were updated by Agshare.Today group using survey data collected in 2017 and are available on the CDP intranet on the Agshare.Today platform.
Objective 3: Characterization of disease vectors	
Whiteflies characterized	• A total of 153 whitefly samples from surveys were analyzed using mt COI primer set and sequence-characterized. • Next-generation sequencing (NGS) datasets (genome/transcriptome) were generated for populations representative of cassava whiteflies of importance in East Africa (SSA1-SG1/SG2, SSA1-SG3 and SSA2). • Comparison of nuclear genes associated with life parameters and fecundity was carried out on eggplant and cassava plants as part of Mr Habibu Mugerwa's (2018) PhD thesis.

Potential insect vectors of CBSVs identified	• In 2016, two transmission trials of Aphid species *Myzus persicae* and *Aphis craccivora* were set up to assess for possible transmission of CBSVs. Preliminary results showed no CBSD infection in cassava after 35 days post-inoculation but Aphid were able to settle in cassava. The experiments will continue when additional funding is secured.
Virus population (species) in whiteflies determined and characterized	• Samples used to develop species delimitation. • Four whitefly samples collected from Tanzania in 2014 were sequence-characterized using NGS and paper published by Sseruwagi et al., (2017) Gates Open Research journal 1:DD16 (DOI) 10.12688/gatesopen res.12783.1.
Objective 4: Diagnostic tools	
ELISA-based tools validated for CMBs and CBSVs diagnostics	• CBSV sequence data used to develop four pairs of peptides: two for CBSVs and two for UCBSVs. • Validation of one peptide for detection of CBSV was successfully completed at TARI–Mikocheni by mid-August 2014 with the technical support of Dr Trino Ascencio (NCSU). • In 2015, four antipeptide antibodies were validated at Julius Kuhn Institute in Germany during a one-month attachment of Dr Fred Tairo. • The four polyclonal antipeptides showed they do bind to CBSVs virions but their antipeptide sensitivity is still low for reliable and specific detection of CBSV. • In 2016, the four pairs of antipeptide were resynthesized to enhance sensitivity. They will be re-evaluated further. • Evaluation of the efficacy of a commercial TAS-ELISA kit for detection and discrimination of CBSVs was completed in 2017 by Mr Dickson Lwabulala and published in his MSc thesis. • Dickson Lwabulala successfully defended his MSc thesis and completed his degree.
Objective 5: Identification of a key gene for resistance-breaking satellites	
Generate a map of chromosome 1 with at least a 200-kb resolution, depending on the spacing of the SNPs	• Strategies were changed to identify the key plant gene responsible for resistance-breaking SEGS. • Generation of a low-resolution QTL map for chromosome 1 was completed.
Identify and test knockout mutants in each of the genes along the 200-kb interval corresponding to the resistance locus (the average gene size in Arabidopsis is ~2 kb)	• A total of 1599 F2 plants were screened for virus symptoms, and genomic DNA isolated from 477 plants that showed minimal symptoms (0–1 on a scale of 0–4). • DNA concentrations were measured from each of these plants and used to make pooled DNA with equal representation from each plant and submitted for sequencing on 2 September 2014. • When the sequencing results are received, bioinformatics will be conducted to identify a small region containing the resistant DNA. It

	is anticipated that 5–10 knockout mutants will need to be tested for verification rather than the 5–100 originally planned. It is hoped to determine the identity of these 5–10 candidate genes.
Aim II: Support clean seed systems for farmers	
Objective 6: Conventional breeding support	
Breeders' material monitored for disease and indexed for virus (CMBs and CBSVs) load at 3, 6, 9 and 12 MAP	• A total of 30 cassava clones (breeding lines) from breeders in Tanzania were screened at TARI–Mikocheni for CMB and CBSVs in 2014. • Of the 30 clones, 11 have been confirmed clean and are maintained in vitro at TARI–Mikocheni. • From 2014 to 2017, four breeding lines were analyzed by MSc student Veneranda Ngazi for their response to CBSVs infection and virus titer quantification. One manuscript is being reviewed by Agshare.Today for resubmission to *Canadian Journal of Plant Pathology*. • Quantification in breeders' materials screened by MSc students and their response to CBSV infection are being evaluated as well as their CBSVs viral loads by qRT-PCR. The results are part of Veneranda Ngazi MSc report. • Veneranda Ngazi successfully defended her MSc thesis and passed; completion awaits acceptance of the manuscript.
Objective 7: Transgenic cassava	
Molecular constructs for CBSV and CMBs resistance developed	• Three molecular constructs, two containing begomovirus replicase genes (EACMV-AC1/5' and EACMV-AC2/AC3) and one with CBSV coat protein, were constructed and cloned into plasmids. • In 2013, two additional molecular constructs: ACMV and EACMV were produced by a PhD student (Elibariki et al., 2014).
Farmer-preferred cassava varieties transformed with RNAi constructs	• TARI-Mikocheni **t**ransformation roofing completed in June 2014. • Farmer-preferred cassava varieties assembled at TARI–Mikocheni were used by Ms Christina Kidulile and two project staff to make at least five constructs, currently mobilized into *Agrobacterium* by August 2014. • MSc student Ms Christina Kidulile attended backstopping training on cassava transformation techniques (friable embryonic callus (EFC) and somatic embryogenesis) at Dr Stephan Winter's laboratory at Julius Kuhn Institute in Germany from September to November 2015. • A cost-effective medium for in vitro propagation of Tanzanian cassava landraces was developed and published (Kidulile et al., 2018). • Focused survey was conducted in November 2015 in Mkinga districts, Tanga region in Tanzania to re-collect the important farmer-preferred cassava landraces and re-establish them in in vitro culture at TARI–Mikocheni.

	• Two local cultivars, Katakya and Paja la mzee, were transformed and are under selection media. They will be moved to maturation. • One MSc thesis completed: Kidulile (2018) MSc thesis, unpublished.
Objective 8: Supporting certification systems	
Cassava materials for certification in TOSCI fields monitored and tested for viruses	• Training of two Tanzania Official Seed Certification Institute (TOSCI) staff (Mr Dickson Lwabulala and Bakari Mrutu) in cassava disease diagnostics completed in 2013 at TARI–Mikocheni. • Mr Dickson Lwabulala (MSc student-TOSCI) successfully completed his MSc on 'Evaluation of serological assays for the detection and discrimination of CBSV and UCBSV infecting cassava'. • In 2013, 27 sources of clean cassava seed were identified by the CDP-MARI team in Kagera and Kisarawe districts for the supply of planting materials for the establishment of primary multiplication fields for the community phytosanitation project led by Dr Kiddo Mtunda. • A total of 4,900 planting materials obtained from cassava multiplication sites, Mkuranga (2000), Bukoba (1150), Chato (950) and from Ifakara-Morogoro (800), were virus indexed at TARI–Mikocheni for the community project. • In 2015 additional 7000 cassava leaf samples from Community phytosanitation plots were indexed for virus.
Field-based diagnostic kits supplied to TOSCI	• Pending completion of validation at TARI-Mikocheni in September 2014. • Validation is in progress using commercially available serology-based kits. Field kits evaluated by MSc student Mr Lwabulala using antibodies from DSMZ. • The kits can detect at least 50% compared to RT-PCR tests. Although lower efficiency, they are more cost effective and thus useful for field detection and quick diagnostic tests.
Objective 9: Reaching farmers directly and through partners	
Farmers trained on CMD and CBSD disease symptom recognition and management strategies	• Six farmer training sessions were conducted at 2, 5 and 8 months after planting (MAP) in Tanzania: five in 2015 and one in early 2016. • A total of 42 farmers from: Rorya (11), Butiama (10) and Mbinga (21) were trained. At 5 MAP, a total of 126 farmers from: Rorya (62), Butiama (64) were trained. • One further training was carried out in Mbinga district in the new demo sites. Fourteen groups (a total of 140 farmers) were trained.
Demonstration plots for benefits of using virus indexed planting materials established on-farm	• First (2 MAP) assessment and training of farmers completed in Mbinga, Butiama and Rorya districts, 4–11 October 2014. • Second (5 MAP) assessment and training of farmers completed in Mbinga, Butiama and Rorya districts, 4–11 October in 2014. • Fourteen new groups were established and provided with improved cassava planting materials to plant 56.6 acres in Mbinga district on 2015.

	• Third (8 MAP) assessment and training of farmers completed in Butiama, Rorya and Mbinga, 11–14 February 2015. • A total of 186,000 cassava cuttings were harvested from the cassava multiplication demos in Butiama and Rorya districts in Mara region in December 2015. • Harvested cuttings were distributed to 36 new farmers groups in Mbinga district, Ruvuma region on 26 December 2015.
Information materials developed and disseminated	• 300 calendars printed and distributed to stakeholders in Tanzania. Approximately 200 reprints of dissemination materials in Kiswahili were distributed to farmers. • 14 project presentations made in district offices, universities and other stakeholders in Tanzania. • A total 340 leaflets were developed, printed and distributed to farmers in the three districts during training on disease identification and in the national agricultural show in Tanzania. • 400 leaflets were reprinted and distributed to agricultural extension officers during training in Mkinga district, Tanga region and farmers in the new groups in Butiama districts, Tanzania in December 2015. • One article on cassava brown streak virus genome published by Ndunguru et al., (2015). • One article on SEGs published by Ndunguru et al., (2016). • In 2017, a total of five papers were published in peer-reviewed open access journals: Reyes et al., 2017, Mbewe et al., 2017, Sseruwagi et al., 2017 and Tairo et al., 2017 and Munganyinka et al., 2017. • In 2018, five papers were published in peer-reviewed open access journals: Sseruwagi et al., 2018, Saggaf et al., 2018, Kidulile et al., 2018, Rajabu et al., 2018 and Boykin et al., 2018.
Aim III: Build sustainable regional capacity	
Objective 10: Strengthening stakeholder linkages	
Disease diagnostic camps conducted	• Could not be achieved due to lack of field kits.
Country Team leaders meeting to develop country-specific milestones	• Milestones agreed for each partner country to work toward.
Project inception and consultative meeting with stakeholders conducted	• Six meetings were held in Dar es Salaam with project stakeholders including: Sugarcane Research Institute, University of Dodoma, University of Dar es Salaam, Sokoine University of Agriculture, Ministry of Agriculture, Agro Biotech (tissue culture laboratory).
Awareness on availability of diagnostic capacities created through	• More than 20 articles published in daily newspapers in Tanzania. • 11 TV programs (Independent television (ITV), Star TV, Tanzania Broadcasting Corporation (TBC) on local TV stations. • Three radio presentations on FM radio stations.

training and different media	• 14 public seminars on the project made in Tanzania and some partner countries during M&E missions. • At these seminars, the CDP project coordinator gave presentations on the role played by CDP in developing virus-resistant cassava using transgenic approaches. These seminars were well attended and the recordings are with the Commission of Science and Technology (COSTECH). • Six radio broadcasts were aired on different topics on cassava virus diseases and the diagnostic project by Dr J. Ndunguru in September 2014 in Tanzania. These were live programs where farmers called the stations with their queries and were provided with advice by experts.
Exchange visits between scientists in the project countries conducted	• The TARI–Mikocheni project team together with Country Project Team Leaders and their assistants participated in the first exchange visit to ZARI, Zambia during 15–21 May 2016. • One of the outreach sites was also visited in Zambia to assess the level of participation of the farmers and the benefits of using virus-free planting cassava planting materials.
Project website established	• Design completed and hosted at www.mikocheni-ari.co.tz. • Project website developed by AgShare.Today.
Access to journals (TEEAL from Cornell University)	• Subscription to American Phytopathology Society Journals obtained in 2014 and this provided access to three journals (Phytopathology, Plant Disease and Plant Microbe Interaction) for all Country Team Leaders. • TEEAL library and video conferencing facilities were installed at TARI–Mikocheni library on 2015 and are now in use.
Outreach to regional virologists in non-project countries	• In 2017, a team of five scientists (Dr Joseph Ndunguru, Dr Fred Tairo, Dr Peter Sseruwagi, Dr Laura Boykin and Ms Jeanine Umufuyisoni) visited Madagascar to establish collaboration with counterparts on cassava virus disease research and management. The team met with the scientists, government and private sector people involved in cassava processing. • One joint manuscript reporting on whitefly in Madagascar was published (Sseruwagi et al., 2018).
Objective 11: Strengthening human capacity and infrastructure	
Human capacity	
Project staff recruited	• Completed in all project countries. A total of 16 project staff recruited on 2014.
PhD and MSc students trained on different aspects of cassava virus diseases	• PhD student Cyrian Rajabu successful defended his PhD thesis in November 2018 and passed. • Six MSc students successfully defended their theses and passed in June 2018.
Advanced specialized training and visits for	• Three project leaders (Dr Joseph Ndunguru, Fred Tairo and Peter Sseruwagi) visited the Agricultural Research Organisation of Israel from 2 to 8 February 2014.

project scientists (1–2 months) conducted	• Mr Charles Kayuki the Lab Manager at TARI–Mikocheni attended a training workshop organized by the International Center for Genetic Engineering and Biotechnology (ICGEB) in collaboration with the National Agency for Biotechnology Development (NABDA)-Nigeria in Abuja for 14 days, from 10 to 23 August 2014. • Dr Joseph Ndunguru and Dr Peter Sseruwagi visited UWA from 16 November to 15 December 2014 and trained on bioinformatics and genomics of cassava viruses and whiteflies. • Dr Fred Tairo visited the Julius Kuhn Institute in Braunschweig, Germany from December 2014 to January 2015. While there, he evaluated the efficacy of four TARI–Mikocheni developed antipeptide polyclonal antibodies for the detection and discrimination of CBSV. He also evaluated the efficacy of one real-time based assay for detection and quantification of virus load in breeders' materials. • Ms Christina Kidulile visited the Julius Kuhn Institute, Braunschweig, Germany for a month to optimize the FEC technique for cassava transformation in 2016. • Ms Esperance Munganyinka a PhD students visited the Julius Kuhn Institute, Braunschweig, Germany for six months to accomplish her PhD research work in 2017.
Extensionists, crop inspectors and other stakeholders (1 week) training	• 51 extension officers were trained on various aspects of cassava virus disease recognition and management in 2014 in Mbinga district. • A total of 56 extension officers in Tanga region were trained on cassava virus disease management in December 2015. • Further training was conducted in October 2014 in Mbinga, Southern Tanzania.
Project staff trained on IP, biosafety issues and communication strategies	• Training in IP and communication strategies was conducted for 17 project scientists during 27–31 October 2014 at the International Centre for Research in Agroforestry (ICRAF), Nairobi, Kenya.
Project results and information disseminated	• 48 scientific papers published in peer-reviewed open access journals during 2012–2018. • 330 project annual meeting proceedings produced and distributed to stakeholders. • 10,000 leaflets, 5000 posters and 300 calendars were produced and disseminated to stakeholders from 2014 to 2017. • CDP project teams participated in 10 annual agricultural shows in Dodoma and Morogoro in Tanzania on 1–8 August 2009–2018 and disseminated info and technologies on cassava viruses. • More than 20 articles published in daily newspapers in Tanzania; 11 TV programs (ITV, Star TV, TBC) on local TV stations; 3 radio presentations and 14 public seminars on the project made in Tanzania.

	• One communication strategy developed and implemented to all project partners by Communications and Advocacy Officer, Mr Greyson Mutembei, in 2014. • Project staff attended nine scientific conferences and presented project results from 2013 to 2018. • Six MSc and 1 PhD theses produced in 2018 are available on the CDP intranet on the Agshare.Today platform. • Nine Annual Project Reports produced and are available on the CDP intranet on the Agshare.Today platform.
Institute Directors trained in leadership and management	• Three project leaders (Dr Joseph Ndunguru, Peter Sseruwagi and Fred Tairo) attended Leadership skills training conducted in July 2014 in Entebbe, Uganda. • Dr Andrew Ngereza, Assistant Officer in Charge, attended leadership skills training in Kigali, Rwanda in 2014.
Infrastructure strengthening	
Diagnostic and virus indexing labs refurbished	• The virus indexing laboratory at TARI–Mikocheni was refurbished and laboratory equipment procured in 2017.
Greenhouses constructed/renovated	• Two screenhouses were renovated in 2017 and are in use.
Vehicles, laboratory equipment and consumables procured	• Two project vehicles procured each in 2009 and 2014 and all are in use. • Various equipment procured and in use.
Project management	
Project management	• A total of three TARI–Mikocheni accountants were trained and provided with the accounting package TALLY, 14–17 May 2013 in Dar es Salaam, Tanzania. • Ms Shubira Katagila (RIP) attended advanced accounting training in New York, USA, in December 2014. • Ms Cecilia Sunga, the Administrative Assistant attended a training course in project management in the UK in December 2014. • A total of three project leaders and one PhD student attended AgShare.Today training and scientific report writing skills in January 2016 in San Diego, USA. • Project coordinator Dr Joseph Ndunguru attended an advanced leadership training in London, UK in 2017.

Background

In Tanzania, cassava is grown on 1,061,043 ha with an average output of 5,575,304 t, which is substantially below Africa's average (10.1 t/ha) (FAOSTAT, 2016). Despite this low productivity, Tanzania accounts for 2% of the world's cassava production; it ranks third in Africa and is among the top 10 world producers of cassava. In Tanzania, cassava is the second most important food crop after maize and supports 84% of the country's estimated 59 million (FAOSTAT, 2016). Cassava is grown mostly by small-scale farmers who own an average farm size of 0.5–10 ha. The producing regions are Mwanza, Shinyanga and Mara regions in the Lake Victoria basin; Kigoma and Tabora regions in the Western zone; Manyara region part of the Northern zone; Morogoro, Tanga, Coast and Dar es Salaam in the Eastern coast; Lindi, Mtwara and Ruvuma in the Southern zone; and the Zanzibar islands (Figure 1). Cassava forms an important component of the cropping system in Tanzania. As in most parts of Africa, cassava in Tanzania is a staple food and raw material for industry and livestock. Recently, in Tanzania, cassava has gained the status of a cash crop due to fresh market sales, and this has stimulated production. Furthermore, cassava is drought-resilient and valuable when other crops fail.

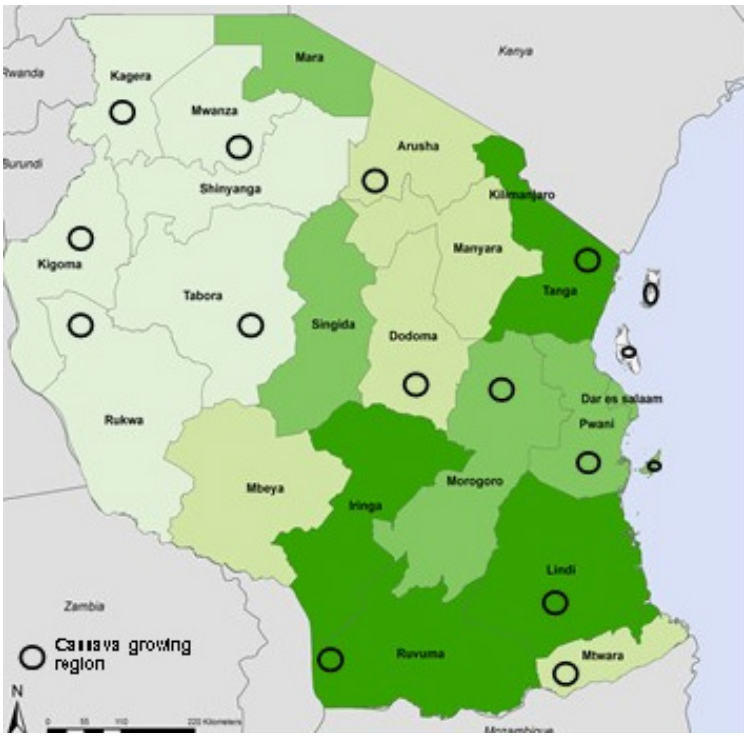

Figure 1 United Republic of Tanzania (Photo source: NBS, 2015), map adapted to illustrate major cassava cultivating regions (circles) within administrative regions (shaded) of Tanzania

Under ideal growing conditions, disease-free cassava can be expected to yield more than 30 t/ha. However, despite attributes such as drought tolerance and low input requirements, yield is still poor in Tanzania at 5 t/ha. This low figure can be attributed to the absence of sound seed delivery systems, despite the deployment of virus-free, improved and superior cassava varieties.

Previous research showed that the prevalence of cassava viral diseases – mainly cassava mosaic disease (CMD) (Ndunguru et al., 2005) and cassava brown streak disease (CBSD) (Mbanzibwa et al., 2009) – contribute more than 70% of yield losses in Tanzania. Their consequences not only result in

reduced crop yields but also undermine the ongoing efforts in genetic improvement for yield, quality and development of virus-resistant cultivars.

Prior to the Cassava Diagnostics Project (CDP) the management of these diseases was inefficient due to low diagnosis and monitoring capability. Such deficiency with respect to plant disease diagnostics not only affects the sustainable management of these diseases but also constrains efficiency of the seed delivery system for certified cassava planting materials. These inadequacies were exacerbated by lack of trained personnel, poor infrastructure, limited coordination and communication among cassava stakeholders, and lack of farmers' awareness of the effects of cassava viral diseases. Several management interventions combining both the multiplication and deployment of virus-free planting materials against cassava viral diseases were executed in several initiatives throughout the country but with limited success.

During 2009–2018, funding from the Bill & Melinda Gates Foundation (BMGF) has contributed to the ongoing efforts to combat cassava viral diseases in the country by enhancing the capability of national cassava research programs (NARS) – through a regional CDP – to diagnose, characterize, monitor and sustainably manage viruses affecting cassava productivity.

The goals of the CDP were tackled in two stages. Phase I (2009–2012) was to enhance the human and infrastructure capacities of partner countries. This would enable us to collectively address cassava viral diseases by using efficient and standardized diagnostic tools. In Phase II (2013–2018), CDP utilized the enhanced capacities built to further augment the capacity of NARS to effectively implement management strategies.

During the two phases of implementation, CDP recorded several achievements: we increased our knowledge on the epidemiology of CMD and CBSD; we progressed in understanding the threat of the evolving viruses and vectors affecting cassava; we supported the initialization of a clean-seed system for the delivery of certified cassava planting materials to farmers by building the human resource capacity of a seed certification agency; and we enhanced farmers' knowledge on the management of cassava viral diseases by demonstrating to them the benefit of using certified virus-tested planting materials.

The CDP also built a sustainable national capability in diagnostics through the short- and long-term training to cassava stakeholders in the country on various aspects of cassava viral disease management.

SECTION ONE: Understanding the threat from evolving viruses and vectors

Disease epidemiology in Tanzania

From the start of CDP, TARI–Mikocheni conducted biannual surveys in all cassava-growing regions in Tanzania. The aim of these surveys was to monitor the prevalence, symptoms and severity of CMD and CBSD, and to assess the whitefly population in the surveyed locations. The data collected were used to generate cassava viral disease and whitefly prevalence maps. These data also enabled the project to identify hotspots and low-disease pressure areas suitable for multiplication and strategic deployment of clean cassava planting materials. The maps generated were shared with cassava stakeholders: District Agricultural Officers, cassava breeders and NGOs involved with agriculture in the surveyed regions. These maps were used to assist decision-making by these stakeholders for cassava viral disease management.

Sampling framework and data collection

The surveys during 2009–2017 covered four agro-ecological zones representing the main cassava-producing areas of the Tanzania mainland: Eastern, Lake Victoria basin, Western and Southern zones. Three survey routes covered 10 regions in 2009 and 16 regions in 2017. The first route surveyed Lake Victoria basin and Western zone; the second route surveyed part of Eastern and Central zone and the third route covered the whole of Southern zone.

In all surveys, a non-uniform sampling design was adopted, which comprised purposive sampling – targeting major cassava-producing districts, random sampling (within targeted districts) and convenience sampling (along major and rural roads) (Bouwmester et al., 2012). In each survey, efforts were made to sample the same fields; where this was not possible, other suitable fields within the same locality were sampled in the same district. Field sampling, CMD and CBSD assessment and whitefly counts were conducted as described in Sseruwagi et al. (2004) and reviewed in the Surveys Manual 2016 (available in the CDP intranet on the Agshare.Today platform).

In the first three surveys – 2009, 2013 and 2015 – data were collected using hard copy survey forms. For the 2017 survey, a different approach was used: the data collection form was reviewed and improved. Then, in collaboration with Cambridge University and Rothamsted in the UK the improved data collection form was converted into an app. This software was uploaded to a tablet and became the preferred vehicle for data collection.

A general linear model was used to analyze data using SPSS v10 (SPSS Inc. Chicago, USA). Mean figures for assessing CMD and CBSD incidence and adult whitefly populations were calculated using unchanged raw data – for assessing symptom severity, the mean was calculated after excluding severity score of 1 (asymptomatic). Pearson's correlation was employed to determine the relationship between altitude and whitefly and CMD incidence. Arch View and Microsoft PowerBI computer software were used to generate GIS and visualization maps for the distribution of cassava mosaic begomoviruses (CMBs), cassava brown streak viruses (CBSVs) and whitefly in the regions surveyed.

CMD incidence and severity

Among the regions surveyed, Kigoma in the Western zone had the highest average CMD incidence of 69% in all surveys, followed by Tanga in the Coast zone with 45.7% incidence in the four surveys. Regions in Central Tanzania, Dodoma and Singida, had the least mean CMD incidence of 6% in the four surveys (Figure 3).

The overall CMD incidence varied between years with the highest incidence being 44.36% in 2009 compared to 25.52% in the 2017 survey (Figure 2). The general trend in the four surveys showed that CMD incidence decreased from 44.36% in Western zone and Lake Victoria Basin to low levels in coastal zones.

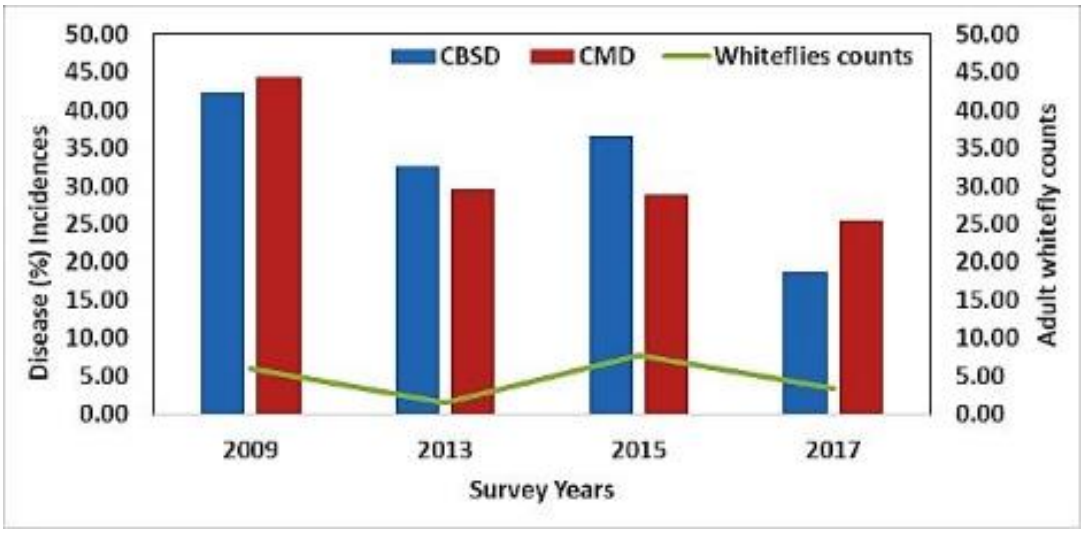

Figure 2 CMD and whitefly abundance trends, 2009–2017 surveys

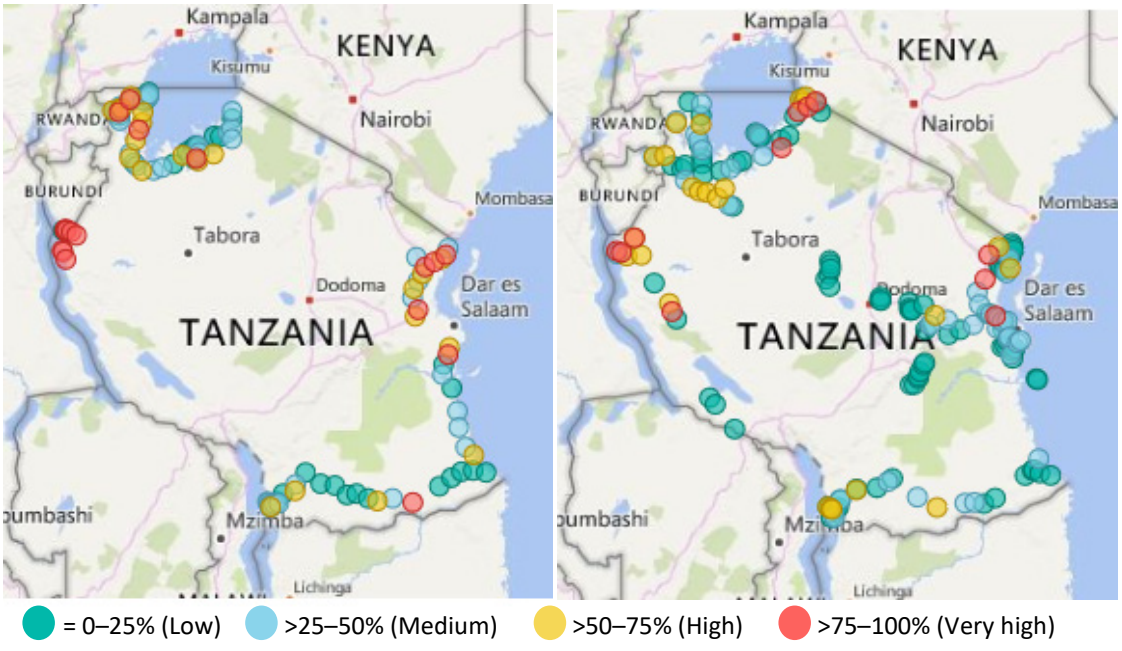

Figure 3 CMD incidence in 2009 (left) and 2015 (right) survey

During our surveys, regions in the Lake Victoria zone (LVZ) – namely Kagera, Geita, Shinyanga, Mwanza and Mara – showed decreasing incidence of less than 50%. These are the main cassava-growing regions and were the regions most affected by the CMD pandemic in the 1990s (Legg et

al., 1998). Regions in Coastal/Southern zone – Coast, Tanga, Lindi and Mtwara – are the second most important cassava-growing areas. Their CMD incidence status fluctuated over the survey years and declined in three regions, except for Tanga which still had a high incidence above 50%.

Generally, CMD symptom severity was mild across regions and years. In most regions, severity ranged within 2.94–3.58, with a mean severity score over the four years of 3.31 (Figure 4).

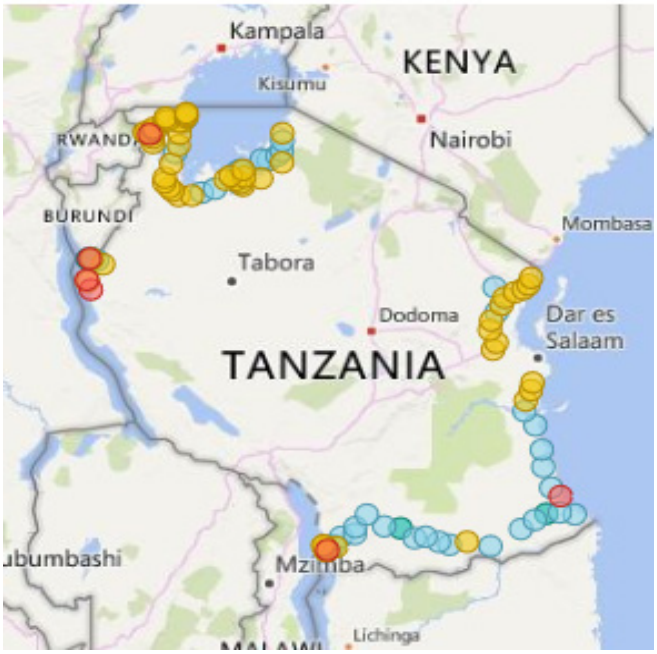

Figure 4 CMD severity map – 2009 survey

Among the regions, the 2015 surveys showed that Mwanza and Mara (both in LVZ) had the highest CMD severity scores of 4.47 and 4.00, respectively. However, the severity scores for the other regions ranged from 3.90 in Kigoma in Western zone to 2.20 in Mtwara in Southern zone (Figure 5).

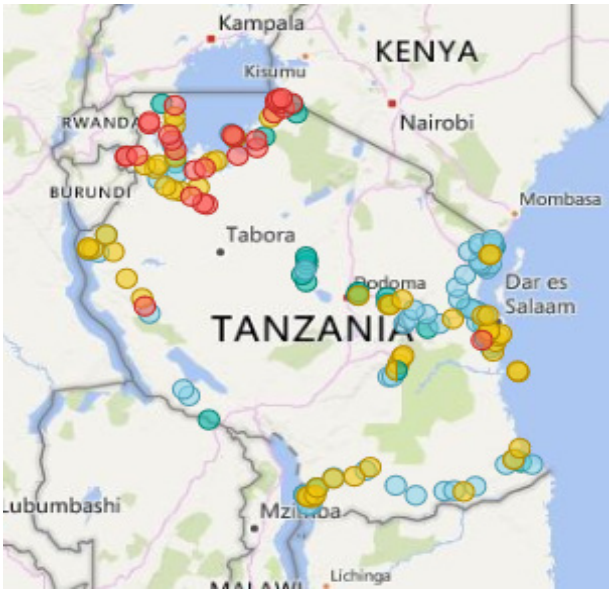

Figure 5 CMD severity – 2015 survey

Whitefly abundance

In all our surveys, the whitefly population abundance was generally low, with an average of 4.75 per plant. The mean number of whitefly/per plant/year ranged from 6.04 (2009) to 1.52 in 2013 surveys. Exceptionally, the highest number of whitefly in the four surveys was in Mwanza (26/plant) in 2017 and (25/plant) in Tanga in 2009 surveys.

CBSD incidence and severity

The overall CBSD incidence in four surveys was 32.2% with highest in 2009 (42%) and lowest in 2017 (18%). The CMD symptom severity was also moderate with score range of 2.3–3.1. The CBSD incidence across agro-ecological zones showed regions in the LVZ still had high incidences of CBSD compared to Coastal/Southern zone regions (Figure 6).

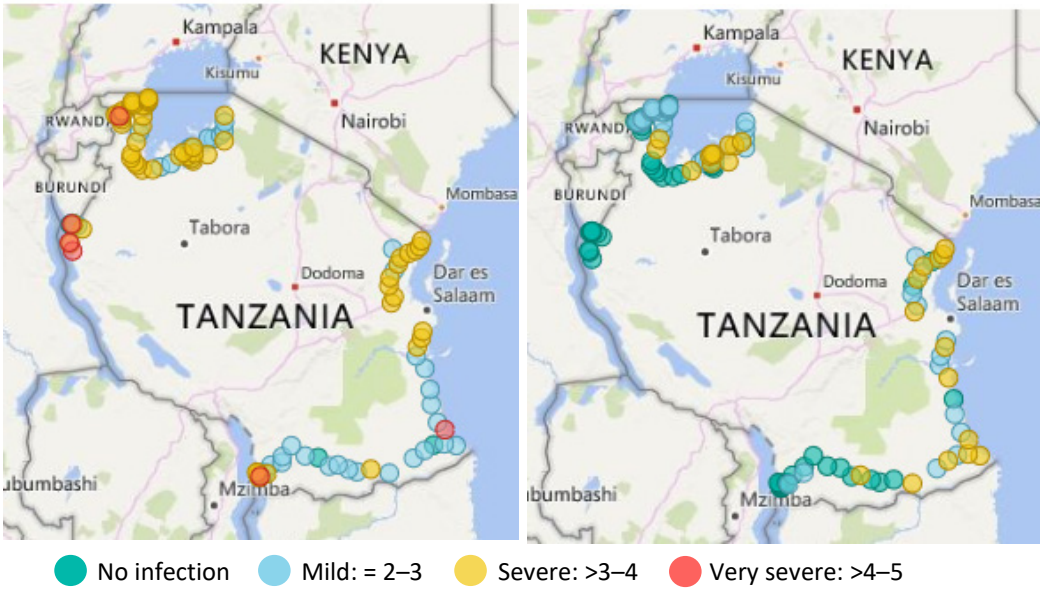

Figure 6 CMD and CBSD severity – 2009 survey

Mara region in the LVZ was the region with the highest CBSD incidence, with high incidence from 2009 (87%) to 2017 (51%) – prevalence of CBSD in the region was also previously reported (Ndyetabula et al., 2017). It is interesting that the CBSD incidence in Mara and Kagera regions, both in LVZ, remained above 50% (Figure 6 and Figure 7) but decreased from 87% in 2009 to 51% in the 2017 survey. This is partly due to the collaborative interventions through the introduction of virus-tested CBSV-tolerant planting materials, coupled with rigorous integrated cassava viral disease management practices employed through farmers' groups in Kagera by the Community Phytosanitation Project led by the Kibaha Sugar Cane Research Institute (SRI) and in Mara by the CDP led by TARI–Mikocheni, both BMGF-funded projects.

The survey results showed that the primary source of infection for both CBSD and CMD was through use of virus-infected planting materials. This called for upscaling of intervention efforts initiated by SRI and TARI–Mikocheni to other cassava-growing regions in order to manage CBSD and CMD prevalence.

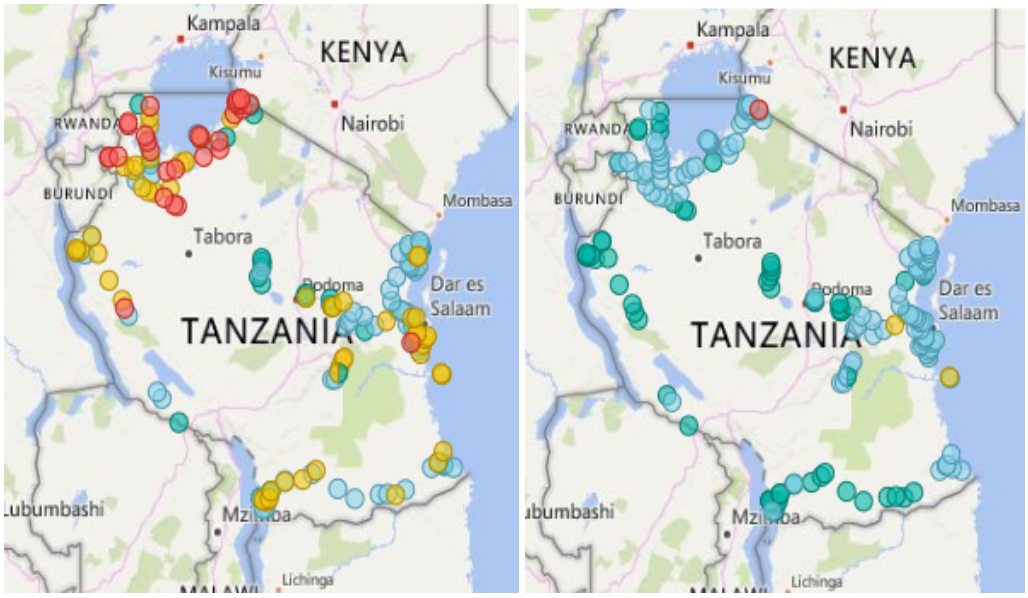

Figure 7 CMD and CBSD severity – 2015 survey

Relationship between CBSV presence/load and disease symptom development determined

Spread of CBSD within and between cassava fields

At the beginning of the CDP project in 2008, knowledge of CBSD epidemiology and the factors influencing disease spread within and between cassava fields was grossly lacking. Thus, field experiments were initiated in Tanzania on spatial and temporal spread of CBSD as an MSc program was undertaken to determine the rate of CBSD spread between and within cassava fields, and the factors influencing disease spread.

Trials were established at Chambezi field station in Coastal Tanzania in a randomized complete block design using virus-free tissue culture raised and RT-PCR checked plants. It contained three treatments: inoculum in the center, inoculum diagonally and inoculum on outer rows to serve as CBSV sources (Gwandu et al., 2015).

The results from two seasons (2012 and 2013) of trials demonstrated that CBSD progressively increased with time and was highly significant for foliar and stem incidence ($P < 0.005$). The effect of spatial arrangement of inocula within the fields was statistically non-significant ($P < 0.005$) but time played an important role in the spread of CBSD. With time, CBSD incidences both foliar and stem increased from 34% to 96% and 2% to 96% during 1–6 months after planting (MAP).

The results also revealed that CBSD spread increased gradually for all treatments, with significant differences at 2 and 3 MAP (Gwandu et al., 2015) between plots with inoculum sources in outer rows. The mean rate of disease spread was 0.90 and 0.744 for foliar and stem, respectively. The results further showed that although the virus was detected during the trial, the source of inoculum was a neighboring old cassava field.

The findings shed light on the nature of CBSD spread within cassava fields and increased the knowledge base of CBSD spread. This is useful for the development of durable management

strategies by different stakeholders. The results also showed that the source of rapid spread beyond 4 months after planting was due to a reservoir of inoculum in neighboring old cassava fields. This suggests that using clean, virus-free planting materials alone might not be an effective solution for CBSD management in areas where the source of inoculum is located close to the new field. Therefore, these findings provide an important contribution when formulating effective integrated pest management strategies for managing CBSD spread within, and into, new cassava fields.

Alternative hosts for CBSVs and CMBs and associated insect vectors identified

Previous studies (e.g. Ndunguru, 2005) suggested that Tanzania is the center of diversity for CMBs. Since 2014, comprehensive studies have been conducted to identify the possible alternative hosts for CBSVs and CMBs in Tanzania in areas where CBSD and CMD were first reported (Storey et al., 1939). In line with biannual disease surveys, samples with virus-like symptoms were also collected from annual weeds and shrubs within cassava fields and their immediate environs. In this activity, Tanzania collaborated with Mozambique, Kenya and Rwanda in searching for alternative hosts for CBSVs and CMBs – through the work of one MSc and two PhD students.

In the 2014 survey, 76 leaf samples with virus-like symptoms were collected in 13 regions from both annual weeds and shrubs in and around cassava fields and screened for presence of both CBSVs and CMBs. An additional survey was conducted on 2015 in the Coastal and Tanga regions where both CBSV and *Uganda cassava brown streak virus* (UCBSV) were detected – using CBSV-specific RT-PCR primers after Mbanzibwa et al. (2011) – in three shrub species *Annona senegalensis* Pers (CBSV), *Solanum incanun* L. (UCBSV) and *Psorospermum febrifugum* (co-infection). The CBSV-positive samples were distributed in the Coast and Tanga Regions (Gwandu et al., 2015).

Similarly, a whole-genome sequence representing DNA-A and -B components was isolated from a non-cassava *Deinbollia borbonica* (Kyallo et al., 2017). Analysis of DNA-A showed that it was closely related to *Tomato leaf curl Mayotte virus* (AM701764; 82%), and the DNA-B shared the highest nucleotide sequence identity with that of *East African cassava mosaic virus* (EACMV, AJ704953) at 65% (Kyallo et al., 2017).

The findings in the search for alternative hosts for CMBs and CBSVs contributed to the epidemiological knowledge gap for both CMBs and CBSVs. They revealed additional diversity and virus-reservoir hosts for both CMBs and CBSVs. Alternative host samples for these viruses were collected from Muheza district (05.10219S, 38.47172E; altitude 202 meters above sea level) at the slope of Usambara Mountains in Tanga, which was previously reported to be the hotspot for cassava virus evolution (Ndunguru et al., 2015) and original place of CBSD (Storey, 1939). The findings also contributed knowledge on the role of alternative hosts in harboring and spreading of CMD and CBSD. This knowledge will form the basis of recommendations for management of both diseases. This is because an efficient polyphagous vector such as *Bemisia tabaci* (Gennadius) moves freely from cassava fields to the surrounding vegetation and the latter may have reservoirs of viruses that can be transmitted to nearby cassava fields.

The RT-PCR detection of CBSV and UCBSV in single and mixed infection in non-cassava species collected from the original place of CBSD justify extension of the comprehensive search for more potential alternate hosts, particularly for CBSD in its place of origin.

Nature of interaction between CBSVs and CMBs/SEGS and its impact on development of disease determined

It has been established under laboratory conditions that CMBs (Ndunguru et al., 2016) and CBSVs synergistically interact perhaps with other molecules (e.g. DNA satellites) to induce CMD and CBSD, respectively (Saggaf et al., 2018). The two viruses commonly occur together in cassava in East and Central Africa. Prior to this project, the nature of the interaction and role of sequences enhancing geminivirus infection (SEGS) with CMBs and/or CBSVs in the disease complex was largely unknown.

Over the past four years (2014–2017), we studied the nature of interaction between CMB *East African cassava mosaic Cameroon virus* (EACMCV) and eSEGS1 (Rajabu et al., 2018) in the model plant Arabidopsis and the interactions between CBSV and eSEGS in *Nicotiana bethamiana* (NB) and cassava – Saggaf (2018) MSc Thesis (unpublished).

The aim of the two studies was to investigate the effect of eSEGS1 and eSEGS2 with CMBs and/or CBSV on disease symptom enhancement and development. The results are reported in detail – Rajabu (2018) PhD thesis (unpublished) and Saggaf (2018) MSc thesis (unpublished).

During experiments the biolistic inoculation of the eSEGS1 and/or eSEGS1 infectious clones into NB graft-inoculated with CBSV revealed higher CBSD incidence (58.3%) than for plants with eSEGS2 (41.6%). In both combinations, although not significantly different, symptom severity increased as time progressed beyond 3 months after inoculation (MAI).

The study of CBSV viral accumulation using qRT-PCR revealed significantly higher titer in cassava plants co-inoculated with eSEGS1 than with eSEGS2. Similarly, CBSV titer in NB was higher, although not significantly, for the eSEGS1 compared with the eSGS2 combination.

Using four model systems – *Arabidopsis* Sei-0, *Arabidopsis* Pla-1, NB plants and *N. tabacum* protoplasts to characterize the impact of SEGS-1 on geminivirus infection – the study showed that SEGS-1 was active in wild-type *Arabidopsis* in co-inoculation experiments with a CMB. A SEGS-1 transgene was also active in *Arabidopsis* inoculated with only a CMB. This study established that SEGS-1 was associated with early symptom development, enhanced disease symptoms, fast disease progression and enhanced accumulation of virus in geminivirus-infected plants.

In situ hybridization studies showed increased numbers of virus-positive cells in plants co-inoculated with ACMV and SEGS-1 compared to plants infected with ACMV alone, indicating that SEGS-1 might trigger a small protein that interacts with a host or viral protein to facilitate or interfere with virus replication, systemic spread and host defenses.

Studies in *N. tabacum* suspension cells further confirmed the impact of SEGS-1 on ACMV infection. Suspension cells co-transfected with ACMV DNA-A and a SEGS-1 clone supported higher levels of accumulation of ACMV DNA-A than protoplasts transfected with ACMV DNA-A alone. This result strongly suggested that SEGS-1 promoted virus accumulation in the absence of systemic movement (Rajabu, 2018).

A similar investigation using *Cabbage leaf curly virus* (CaLCuV) showed that this virus could also infect a resistant *Arabidopsis* accession Pla-1 when co-inoculated with SEGS-1, indicating that SEGS-1 also broke resistance in *Arabidopsis*.

Our research demonstrated that a combination of eSEGS1 with CBSV or ACMV phenotypically induced severe CMD and/or CBSD symptoms and elevated respective virus titers compared with co-infection with eSEGS2, suggesting that eSEGS1 synergized with both CMBs and/or CBSV to induce severe CMD or CBSD infection, respectively, in both NB and *N. tabacum.* These observations correspond to our previous work (Ndunguru et al., 2016), which also demonstrated that eSEGS1 enhanced CMD symptoms in TME 3 landrace with elevated CMD symptom severity and EACMV-UG viral DNA titer. The results of these two studies shed further light on potential breeding strategies for virus-resistant cassava material against CMD and CBSD. Future studies must address the mechanisms underlying resistance breaking and determine whether similar or different mechanisms overcome *Arabidopsis* Pla-1 resistance versus CMD2 resistance in cassava.

Characterization of emerging viruses

Cassava virus isolates in Tanzania sequenced and analyzed

In Phase I (2009–2013), a diagnostic tool for routine simultaneous detection of four CMD-causing begomoviruses (Aloyce et al., 2013) was developed and validated using virus isolates from Tanzania and published sequences. This development was made possible using a conventional sequencing method. In Phase II, further improvement of diagnostic tools for CBSD was achieved through use of sequence information from existing and new emerging viruses characterized from the 2013 and 2015 country surveys.

In 2013, 12 new whole-genome sequences were sequenced and analyzed: five CBSV and seven UCBSV (Ndunguru et al., 2015). Sequence information showed that both CBSV and UCBSV were widely distributed in different agro-ecological zones in Tanzania: low, medium and high altitude at >1000 m above sea level. This contrasted with previous reports (Alicai et al., 2007; Mbanzibwa et al., 2009) that CBSV occurrence was limited to low and medium altitude areas below 1000 m, while UCBSV occurred in the highland areas (>1000 m) of East Africa. The results revealed the existence of two further species (CBSV and UCBSV) and more diversity in UCBSV with three more clades (Figure 8). These findings were also demonstrated by Mbewe et al. (2017) using 16 additional CBSV isolates collected in 2016 in Bagamoyo, Coast region.

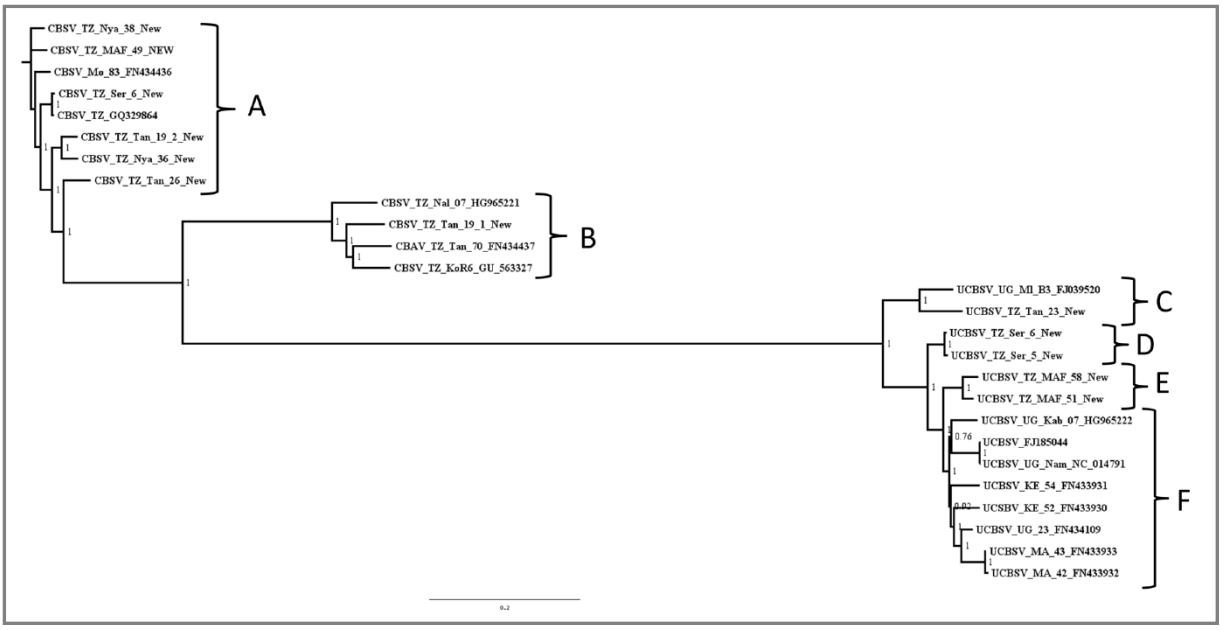

Figure 8 Whole CBSV/UCBSV genomes (nt) analyzed revealed existence of another subclade B with predominantly CBSV isolates from Tanzania (adapted from Ndunguru et al., 2015)

With respect to CMBs, an additional focused survey was conducted on non-cassava producing regions with potential for cassava cultivation in 2016: 13 complete DNA A component sequences were isolated from *Manihot carthaginensis* subsp. *glaziovii* (Müll. Arg.) Allem and sequenced-characterized (Tairo et al., 2017). The results showed 96–100% nucleotide sequence identity with EACMV isolates from Kenya and that they phylogenetically belonged to a single CMB species EACMV monophyletic clade that was distinct from all other CMB species (Tairo et al., 2017).

In four years, the project significantly contributed to the virus GenBank (https://blast.ncbi.nlm.nih) by depositing a total of 13 and 28 CMB genome sequences from wild cassava relatives and CBSVs, respectively. The information on molecular resolution of genetic diversity of CBSVs (Ndunguru et al., 2015; Alicai et al., 2016) showed that the current diagnostic primers for CBSVs (Mbanzibwa et al., 2011) are insufficient to provide confident and comprehensive diagnosis of these viruses and so there may be as many as four distinct virus species that the current primers may miss during screening of planting materials.

Thus, the information enabled the designing of new primers (primer list on CDP intranet on the Agshare.Today platform) on a common CI genomic region of all CBSVs (Ndunguru et al., 2015) that can comprehensively screen cassava planting materials for CBSV infection for certification.

Cassava virus-distribution maps (incidence, severity, whitefly, viruses and eSEGS)

Mapping the distribution of cassava viruses in the country is important for understanding the actual status and identity of prevalent viruses. This step contributed to the management of infestation and of further virus spread into new areas. The information also determined where investment on commercial cassava cultivation should focus.

From 2013, we generated virus-distribution maps using georeferenced coordinates of the cassava viral disease-sampled locations and laboratory virus results of the representative virus isolates collected from the country-wide surveys. Maps for CMBs and CBSVs were generated using GIS/ArcView software. These maps differentiated cassava viral diseases (ACMV, EACMV and

EACMV–ACMV co-infections) and CBSVs (CBSV, UCBSV and UCBSV–CBSV co-infections). The virus-distribution maps were reviewed biannually and updated with current information from the respective surveys of 2013, 2015 and 2017. In 2014, a total of 85 cassava viral disease and virus-distribution maps (Figure 9) were distributed in 20 district agricultural offices and other cassava stakeholders in four regions in the LVZ.

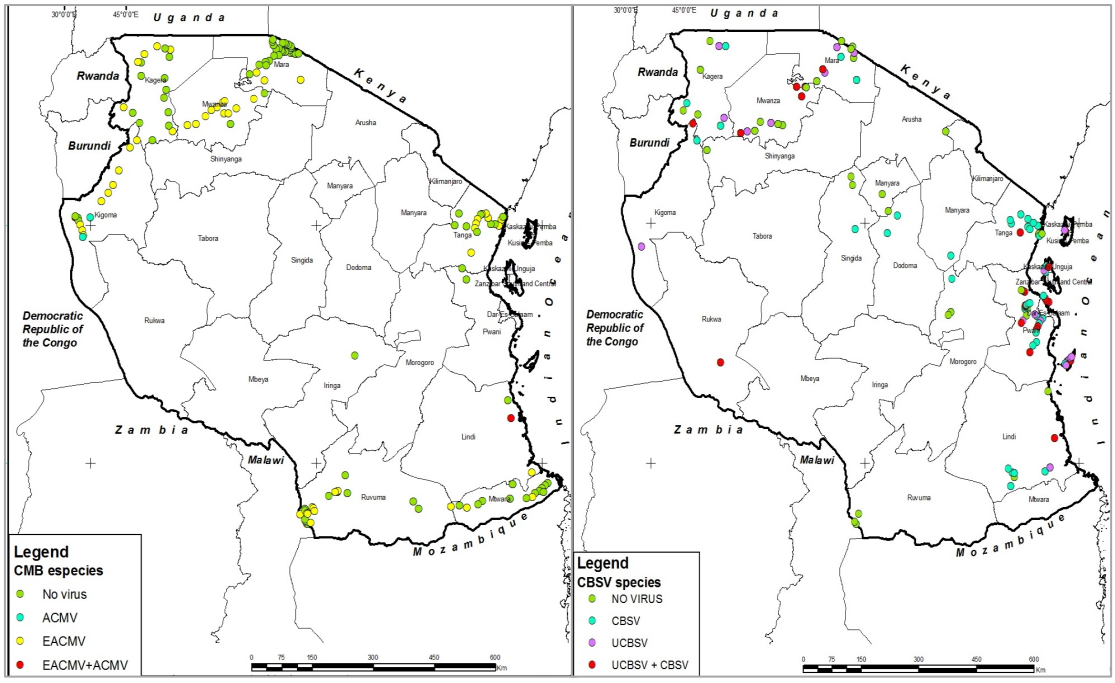

Figure 9 Distribution of viruses affecting cassava in Tanzania: left (CMBs) and right (CBSVs) from the surveys 2013

The distributed maps were accompanied with hands-on instructions on how to use them and were instrumental for guiding cassava stakeholders on where the virus hotspots are and indicating the best areas for setting up trial sites, multiplication sites for cassava planting materials and where to deploy virus-resistant materials for dealing with the prevalent virus for a given location.

Characterization of disease vectors

Whiteflies characterized

The cassava whitefly *B. tabaci* is a major vector of plant viruses that cause the two ongoing cassava mosaic diseases: CMD and CBSD (Maruthi et al., 2005). Apart from transmitting disease-causing viruses, the insects can also cause up to 40% yield loss through direct feeding and from sooty molds (Omongo et al., 2012) that grow on whitefly-produce honeydew, thus reducing plant photosynthesis and by extension, tuber yield.

Thus, in order to develop novel whitefly-management technologies and to ensure their successful adoption by resource-poor farmers, knowledge of the prevalent species and/or biotype is vitally important to aid development of durable and specific management strategies including resistant cassava varieties against known whitefly. From 2013 to 2017 – during the biannual virus surveys – TARI–Mikocheni also collected representative samples in order to determine the population and identity of *B. tabaci* in cassava fields in Tanzania. Adult whitefly samples were collected from the abaxial surface of cassava leaves (Figure 10).

Figure 10 (left) Dr Sseruwagi collecting whitefly on cassava; (right) Abaxial surface of cassava leaf showing whitefly infestation

Using molecular techniques described in Mugerwa et al. (2012), representative whitefly samples from the surveys were sequence-characterized using the *mitochondrial cytochrome-oxidase 1* gene (*mtCOI*) gene. The analysis showed that two *B. tabaci* species occurred on cassava in Tanzania: Sub Saharan Africa (SSA1) and Indian Ocean. The predominant SSA1 had four distinct subspecies groups: SG1, SG2, SG3 and SG4 (Mugerwa et al., 2012).

Further analysis revealed that SSA1-SG1 was dominant and together with SSA1-SG2 was predominant in the LVZ and north-eastern Tanzania, respectively, and SSA1-SG3 was predominant in Coast zone (Sseruwagi, unpublished). The analysis also revealed that SSA1-SG4 is a unique species occurring only in Mara region in the LVZ in Tanzania. The results on whitefly characterization enhanced knowledge on the identity and distribution of whitefly species present in Tanzania and contributed to the breeding of whitefly resistance currently performed in the region.

Potential insect vectors of CBSVs identified

Until now the only known potential vectors of CBSVs were *B. tabaci* (Maruthi et al., 2005) and spiraling whitefly (*Aleurodicus dispersus*) (Mware et al., 2009). However, two reports showed that field transmission of CBSVs by *B. tabaci* took place at low efficiency of about 20% and 25%, respectively, or was sometimes unsuccessful (Holling et al., 1976) and so could not to be definitively associated with the observed rapid CBSD spread within and between fields or the escalating regional epidemic.

Several studies (e.g. Legg et al., 2014) correlated high whitefly abundance with high CBSD incidence in the field, although some did not find this to be the case. This has led to the suggestion that there may be another vector in addition to *B. tabaci* involved in transmitting CBSVs.

Recently, Ateka et al. (2017) uncovered the aphid transmission-associated DAG motif within coat protein (CP) genes of all CBSV whole-genomes at amino acid positions 52–54, but not in UCBSV. Upon further investigation, the DAG motif was also found at the same positions in two other Ipomoviruses – *Squash vein yellowing virus* and *Coccinia mottle virus* – increasing suspicion that aphids could be vectors of CBSV.

Following the above work by Kenyan CDP scientists, TARI–Mikocheni initiated transmission studies to determine if aphids could pick up and transmit CBSVs from CBSD-infected leaves to virus-free cassava plants. The experiment comprised three inoculum sources: cassava plants singly infected

with CBSV or UCBSV and dually-infected with CBSV+UCBSV. Two separate colonies of aphids, *Myzus persicae* and *Aphis craccivora* (groundnut aphid), were established using field-collected, 4th-instar nymphs and wingless adults from okra plants and cowpeas. These colonies were maintained on okra plants in insect-proof cages. The transmission experiments were carried out following TARI–Mikocheni's standard protocols (unpublished). Using RT-PCR, the presence of CBSVs in cassava cultivar Kibandameno was confirmed in both the inoculum-source and the virus-free plants (unpublished, Transmission Report, CDP intranet on the Agshare.Today platform).

Preliminary results showed no CBSD infection in cassava after 35 days post-inoculation. Interestingly, adult aphids were able to settle on cassava plants and laid eggs within the first five days of the experiment. Further experiments are required to test as many aphid species as possible, before definitive conclusions can be reached on aphid transmission of CBSVs.

Virus population (species) in whiteflies determined and characterized

At present, whitefly species identification and the virus populations (virome) they carry is based on the partial sequence of *mtCOI* using DNA extracted from the pool of whiteflies collected from one site, which are regarded as one population. This procedure has several drawbacks, the most serious that *mtCOI* is a mitochondrial marker and hence inherited maternally but it is also difficult to associate the isolated virus species with a given whitefly species when the template DNA is extracted from the pooled whitefly samples.

Thus, a key step in the development of accurate, robust and easy-to-use diagnostic tool that can identify the virus population within whitefly bodies is a method capable of extracting DNA from a single whitefly.

Thus, for genetic identification of whitefly, TARI–Mikocheni developed and optimized an RNA extraction protocol that extracts RNA from a single whitefly and used this to sequence the transcriptome of four individual adult SSA1 *B. tabaci* from Tanzania. Transcriptome sequencing resulted in 39–42 million raw reads. The de novo assembly of trimmed reads yielded 65,000–162,000 transcripts across *B. tabaci* transcriptomes (Sseruwagi et al., 2018).

Bayesian phylogenetic analysis of *mtCOI* sequences grouped the four whiteflies within the SSA1 clade. The BLASTn search (*https://blast.ncbi.nlm.nih.gov/Blastn.cgi*) on the four transcriptomes identified five endosymbionts – the primary endosymbiont *Portiera aleyrodidarum* and four secondary endosymbionts: *Arsenophonus, Wolbachia*, *Rickettsia* and *Cardinium* spp. – that were predominant across all four SSA1 *B. tabaci* samples with prevalence levels of 54.1–75.0% (Sseruwagi et al., 2018).

Amino acid alignments of the *NusG* gene of *P. aleyrodidarum* for the SSA1 *B. tabaci* transcriptomes of samples WF2 and WF2b (Sseruwagi et al., 2018) revealed an 11-amino-acid residue deletion that was absent in samples WF1 and WF2a. Comparison of the protein structure of the NusG protein from *P. aleyrodidarum* in SSA1 with known NusG structures showed the 11 residue (Sseruwagi et al., 2018) deletion was found in a loop region that is variable in length and structure across bacterial species, but absent from Archaeal and Eukaryotic species.
The use of field-collected specimens means time and money will be saved in future studies using single whitefly transcriptomes in monitoring vector and viral interactions. Our method is applicable to any small organism for which RNA quantity has limited transcriptome studies.

Diagnostic tools

Laboratory-based diagnostic tools developed

In Phase I, MSc research at TARI–Mikocheni resulted in development of two diagnostic methods for simultaneous detection and discrimination of four CMBs. The developed tools entailed a single-tube duplex and multiplex PCR (*m*PCR) for simultaneous detection of four CMB species in cassava plants (Aloyce et al., 2013) where diagnostic multiplex primers were developed and protocol for extraction of quality RNA from herbarium plants was developed to simplify surveys and sampling in remote areas without depending on cool storage. The discrimination of the four CMBs was further simplified by development of simple reliable RT-PCR/RFLP methods that discriminated among the four CMBs using restriction enzymes (Rajabu, 2014). The developed diagnostic tool for CMBs was further validated by MSc research student Mr M. R. Mulenga).

In Phase II, TARI–Mikocheni continued to refine more lab-based virus diagnostic tools for CBSD using virus sequence data obtained from the country-wide surveys of both Phase I and II. Analysis of 12 new complete genomes (Ndunguru et al., 2015) enabled redesigning of a new diagnostic primer pair using a sequence of the CI region of the genome which is more stable than the previous genomic regions (Abarshi et al., 2010; Mbanzibwa et al., 2011) which were unable to detect some species clades.

Validating field and lab-based diagnostic tools for CMBs and CBSVs

ELISA-based field kit

In line with the development of lab-based diagnostics tools, TARI–Mikocheni aimed to develop an enzyme linked immunosorbent assays (ELISA)-based field kit to enhance the capacity of cassava stakeholders including regulatory agency Tanzania Official Seed Certification Institute (TOSCI) for seed certification, NGOs for multiplication of virus-free planting materials, breeders and other cassava projects within the region. As part of an MSc program (Lwabulala, 2018), four pairs of CBSV-antipeptide antibodies were developed and evaluated to form a field base kit. In addition, the efficacy of commercial triple antibody sandwich (TAS)-ELISA kit developed by Deutsch Sammlung von Mikroorganiesm und Zellkulturen (DSMZ), Germany, was evaluated for its efficacy in detecting and discriminating CBSD-causing viruses.

Using a total of 150 CP sequences of CBSV and UCBSV obtained from isolates collected during 2009–2013 surveys in Tanzania analyzed together with published sequences in the NCBI database, four pairs of antipeptides – two each for CBSV and UCBSV – were synthesized and their efficacy evaluated at Julius Kuhn Institute, Germany. Evaluation showed that they were capable of detecting CBSV virions, but with sensitivity too low for reliable and specific detection of CBSVs. They were resynthesized in order to enhance their sensitivity and re-evaluated again at North Carolina State University (NCSU) by Dr Trino Ascencio-Ibáñez.

Similarly, the efficacy of a TAS-ELISA kit with monoclonal antibodies (MAbs) and mixed antibodies for CBSD-infecting viruses developed by DSMZ was evaluated (Lwabulala, 2018, MSc Thesis unpublished) to determine sensitivity and specificity in detection and discrimination of CBSVs and cost-effectiveness for routine use in certification of cassava planting materials. The results showed that although the gold standard RT-PCR had more sensitive (100%) detection using screenhouse plants, the MAbs for CBSV and UCBSV were more sensitive (60% and 59.9%), respectively, in co-

infected cassava leaf samples. The specificity was 100% for correct detection of each virus. TAS-ELISA is more cost effective (US$452.98/100 samples) compared to RT-PCR (US$558.98/100 samples).

The validation also determined that the lower mature leaf was the best sampling position for effective and reliable detection of both CBSV and UCBSV in cassava planting materials. However, hitherto no single detection tool has universally been recognized as a standard tool for routine screening of certified planting materials, so the efficacy and cost effectiveness shown by TAS-ELISA compared with RT-PCR make it suitable for routine use in combination with RT-PCR in screening of large samples for certification schemes.

Identification of a key gene for resistance-breaking satellites

Since 2013, the research group led by Prof. Niki Robertson and later Dr Trino Ascencio-Ibáñez (in collaboration with Prof. Linda Hanley-Bowdoin at NCSU, USA) has been working on identifying a plant gene that protects against the disease-enhancing symptoms caused by satellite-like molecules. This work, funded by CDP, was carried out in an *Arabidopsis* plant system in which the satellite-like molecule symptoms could be clearly studied. This was not possible in cassava due to the complication of integrated satellite-like sequences in the cassava genome.

The NCSU group made substantial progress toward mapping a quantitative trait locus (QTL) responsible for resistance to satellite-like molecules in the *Arabdopsis* ecotype resistant line Pla-1. In their work they continued to finish the mapping using a combination of high-density single nucleotide polymorphism mapping of the $F_{2:3}$ population and genome wide association studies.

Impressive progress has been published (Reyes et al., 2017). Responses of virus-induced gene silencing (VIGS) using a geminivirus vector have shown surprisingly diverse responses in 190 *Arabidopsis* accessions. Only the Pla-1 accession was resistant to VIGS, and to three diverse wild-type geminiviruses. Mapping studies revealed a novel recessive locus that, once identified, could bolster breeding efforts for resistance against these devastating DNA viruses. Broad-based immunity is necessary to combat the high rate of geminivirus evolution and the frequent occurrence of mixed infections.

The novel findings of Reyes et al. (2017) accelerated the research efforts and significant progress has been registered in several activities in this work:

- A map created of the CaLCuV QTL in chromosome 1 with at least a 200-kb resolution using KASP genotyping and QTL-Seq
- Generated near isogenic lines to validate our putative QTL
- Complementation of the resistant line (Pla-1) with a Col-0 allele to restore geminivirus susceptibility.

Although this work extends beyond the CDP period which covers the project, it will benefit the future search for resistance to CMBs.

SECTION TWO: Integrated pest management

Conventional breeding support

Breeders' material monitored for disease and indexed for virus (CMBs and CBSVs)

To ensure effective breeding for durable resistance, in Phase I the project collaborated with cassava breeders in Tanzania to enable them to generate virus data along with breeders' materials, and to make informed decisions on the selection of parent and segregating populations. Joint meetings with breeders in the country were conducted in 2011 (in Zanzibar) and with regional virologists and cassava breeders in 2015 in Kunduchi, Dar es Salaam, Tanzania to agree on common procedures in evaluation of cassava virus resistance. During discussion, standard procedures for disease monitoring (Hahn et al., 1994), virus testing and viral load quantification were re-evaluated and agreed for adoption by both virologist and breeders.

Several virus disease-monitoring surveys were conducted in Tanzania in breeders' trials (Figure 11) and a total of 30 clones were indexed for CMD and CBSD and 11 confirmed clean. Representative copies of the virus-free clones are maintained in vitro at the TARI–Mikocheni tissue culture laboratory.

Figure 11 Kizimbani breeding station, Zanzibar: virologist Dr Joseph Ndunguru (third right) explaining to breeders how to score disease

Support to cassava breeders

From 2015 to 2017, four advanced breeders' materials including two improved materials resistant to CBSD KBH/2002/135 and KBH/2000/02 and two controls Kiroba (CBSD resistant) and Albert (CBSD susceptible) were evaluated for their response against CBSD infection (Figure 12), and viral load was quantified to determine the nature of response against CBSD infection as part of MSc studies (Ngazi, 2018 unpublished).

Figure 12 (left) Ms Ngazi, MSc student, graft-inoculating cassava breeders' materials with CBSV inoculum (scion); (right) graft-inoculated cassava plants in screenhouse at Kibaha SRI, Tanzania

Results showed CBSD incidence and symptom severity were increasing with time at 3 and 6 MAI. Similarly, relative virus load was more pronounced at 6 MAI. CBSV severity and accumulation was higher than for UCBSV. Of the evaluated varieties – on a scale of 1 to 5 – KBH 2006/026 had lower CBSD symptom severity (2.07 and 2.38) and virus accumulation (0.04- and 0.71-fold) than other varieties (Table 1) at both 3 and 6 MAI. However, the results showed that CBSD symptom expression did not always correlate with virus load, e.g. Kiroba had higher UCBSV mean severity score (2.4 and 3.2) compared to CBSV and double infection but maintained low UCBSV load of 0.56- and 1.66-fold for 3 and 6 MAI, respectively. Similar trends were also observed in variety KBH135 with slightly higher CBSD symptom severity (2.22 and 2.84) but maintained low UCBSV load (0.43 and 1.01). Table 1 below shows that varieties KBH 2006/026 and KBH 2002/135 responded differently to CBSV infections.

Table 1 Mean relative virus RNA titer of all cassava varieties bred for CBSD resistance at 3 and 6 months after inoculation in the screenhouse

Treatment	Genotype	Mean relative viral RNA	
		3 MAI	6 MAI
Health	Kiroba	0.0	0.0
	KBH 2006/026	0.0	0.0
	KBH 2002/135	0.0	0.0
	Albert	0.0	0.0
CBSV	Kiroba	0.06	0.8
	KBH 2006/026	0.04	0.71
	KBH 2002/135	0.55	1.22
	Albert	8.94	13.8
UCBSV	Kiroba	0.56	1.66
	KBH 2006/026	0.01	0.36
	KBH 2002/135	0.43	1.01
	Albert	2.19	5.28
Double infection	Kiroba	0.03	0.3
	KBH 2006/026	0.02	0.12
	KBH 2002/135	0.05	0.59
	Albert	0.46	1.11
Mean		**1.11**	**2.24**
SE		**1.346**	**3.124**
CV (%)		**21.1**	**39.1**

Treatment	Genotype	Mean relative viral RNA	
L.S.D		1.712	1.987
F-prob		<0.001	<0.001

Source: Ngazi (2018) MSc Thesis (unpublished)

Transgenic cassava

Since Phase I started in 2008, TARI–Mikocheni has been developing its capacity in genetic engineering to improve resistance of farmer-preferred cassava clones against two serious diseases of cassava: CMD and CBSD. The approach taken was to transform farmer-preferred varieties with local virus species to confer resistance against both diseases. The infrastructure capacity was significantly strengthened in Phase I to address this type of work and included establishing a complete, functional Biosafety Level 2 containment facility and obtaining appropriate permits for carrying out contained cassava transgenic work at TARI–Mikocheni.

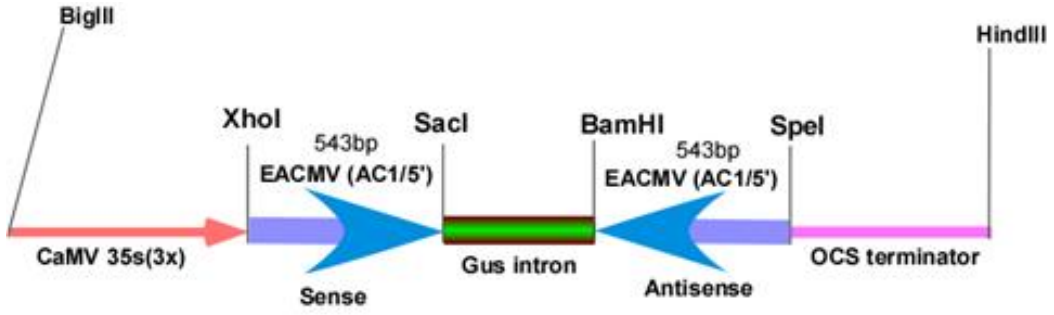

Figure 13 Structure of the molecular construct for EACMV carrying two genes used for cassava transformation to confer resistance against EACMV

The transformation work started with the construction of three molecular constructs in plasmid (Figure 13), which contained begomovirus replicase genes of EACMV-AC1/5' and EACMV-AC2/AC3, and CBSV CP, expressed in sense and antisense, which confer resistance to CMBs and CBSVs, respectively, through a RNA gene-silencing approach. The constructs where immobilized into *Agrobacterium* before transformation. In Phase II in 2013, two additional molecular constructs, ACMV and EACMV, were produced by a PhD student (Gladness Elibariki) – partially supported by CDP (Elibariki et al., 2014).

Induce cassava embryogenic cultures

In transformation of cassava, TARI–Mikocheni pursued two protocols for inducing embryonic cultures: friable embryonic callus (FEC) and somatic embryogenesis. Following several optimizations and technical support from ETH Zurich and NCSU in the USA, the somatic embryogenesis protocol was optimized. Thus, in Phase II, 11 cassava landraces were selected from among the collection of country surveys in Tanzania 2009 and 2017 for somatic embryo induction. Of these 11 landraces, only four were used for transformation work based on their rate of producing embryos. The four selected landraces produced 10 embryos per plant within four weeks and were regenerated into complete plants.

Similarly, our FEC protocol was optimized with assistance from Julius Kuhn Institute, Germany. This enabled us to produce somatic embryos from two cultivars (Figure 14) – TMS and Albert – using immature leaf lobes. However, TMS had a higher number of somatic embryos than Albert.

Transforming farmer-preferred cassava varieties with RNAi constructs

Prior to actual transformation with virus genes, the protocol was optimized by transforming local cassava landraces (Katakya and Paja la Mzee) with a *GUS* construct – a reporter gene. The transformation was performed on both somatic and FEC calluses, co-cultivated with *Agrobacterium tumefaciens* strain LBA 4404 constructs.

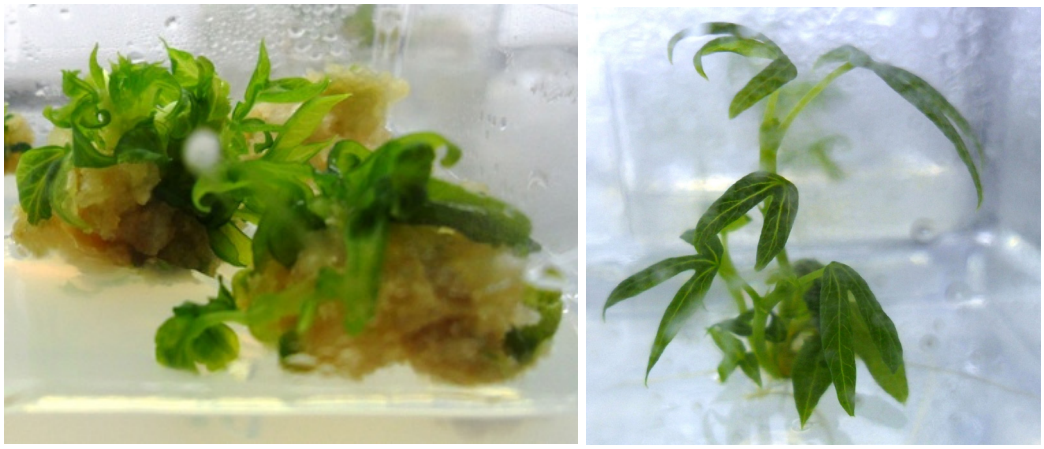

Figure 14 (left) TMS 6044 cassava plants emerging from *GUS*-transformed FEC; (right) TMS 6044 complete cassava plants

Create awareness on biotechnology

At the beginning of the project in 2013, genetic engineering technology was in its infancy in Tanzania. Government and general public awareness of genetic engineering was equally low, which led to establishing regulations unfavorable to science advancement. Thus, in Phase I of the project, TARI–Mikocheni – in collaboration with the Commission of Science and Technology (COSTECH) of Tanzania, the Ministry of Agriculture and biotechnology stakeholders – actively participated in awareness-creation campaigns on the safe use of biotechnology. This resulted in the reviewing of prohibitive regulations of strict liability on GMO technology. Significant efforts were made by TARI–Mikocheni and other stakeholders, including preparation of government cabinet papers in early January 2012, to review the regulatory environment including the removal of prohibitive clauses. TARI–Mikocheni also conducted three awareness-creation open-forum presentations on cassava transformation organized by COSTECH and the African Agricultural Technology Foundation, which resulted in four articles in public newspapers (CDP intranet on the Agshare.Today platform), three radio programs on science and technology, two TV programs on science and technology, and four presentations to government officials and the general public during three national agricultural shows.

TARI–Mikocheni also hosted His Excellency the President of the United Republic of Tanzania, the Honorable Jakaya Mrisho Kikwete, when he officially visited the Institute and inaugurated the transformation laboratory (Figure 15).

Figure 15 (left) His Excellency the President inaugurating the genetic transformation laboratory at TARI–Mikocheni in 2013; (right) H.E. the President sharing his ideas with TARI–Mikocheni scientists after receiving a briefing on the ongoing cassava transformation work

Farmer-preferred cassava cultivars transformed with RNAi constructs

The actual transformation of the four landraces that successfully produced embryos faced some technical challenges. The transformed cotyledons were expected to regenerate into complete new whole plants but this stage of regeneration failed and the cotyledons died. However, calluses on the control plate developed well. TARI–MIKOCHENI is continuing to optimize the transformation protocol and hence produce transgenic plants and thence evaluate their virus resistance prior to application for a confined field trial permit.

Supporting certification systems in Tanzania

Cassava materials for certification in TOSCI fields monitored and tested for viruses

Prior to CDP the system for the delivery of virus-tested clean planting materials for cassava was non-existent due to TOSCI's low capacity to support the delivery system. This low capacity entailed limited human resource in virus detection, infrastructure capacity and guidelines for testing root and tuber planting materials for viruses and certification.

Since 2013, CDP has supported TOSCI and cassava breeders in ensuring delivery of virus-tested planting materials through monitoring of cassava multiplication fields and testing representative samples for viruses CMD and CBSD infection for different cassava projects, e.g. cassava phytosanitation project and Cassava Varieties and Clean Seed to Combat CBSD and CMD project (5CP). In 2013, using a standardized procedure for CBSD disease assessment and sampling (Great Lakes Cassava Initiative Project Manual, 2008), TARI–Mikocheni enabled identification, screening and mapping of clean sources of virus-free cassava planting materials from 27 cassava seed sellers in Kagera and Kisarawe districts and advised the Community Phytosanitation Project on the establishment of primary multiplication fields (Figure 16).

In 2014 and 2015, following establishment of primary cassava multiplication fields in Kagera district, CDP conducted routine CMD and CBSD inspection at six-month intervals – in total 7000 samples were analyzed. From the laboratory results, CDP advised the Community Phytosanitation Project that was being executed by Kibaha SRI on where to deploy the materials in districts. Qualifying districts were those where CBSD infection was already present, but which maintained infection at a low level through rigorous roguing of symptomatic plants.

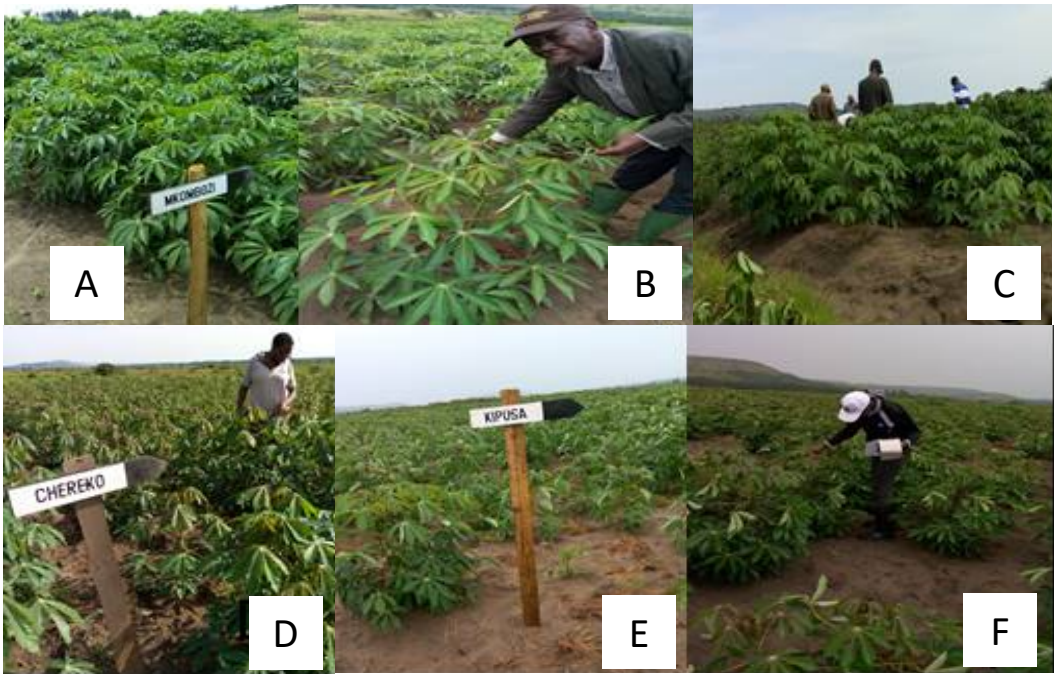

Figure 16 Monitoring and mapping CBSD in multiplication fields in Chato, Muleba, Kisarawe and Mkuranga districts

Training TOSCI staff

One way that CDP supported TOSCI was through building of human resource capacity in disease diagnostics. This support included disease monitoring, sampling techniques, sample processing and virus screening using ELISA and PCR procedures. In 2013, CDP TARI–Mikocheni trained two TOSCI staff: MSc student Mr Dickson Lwabulala registered at Nairobi University of Kenya and MSc student Bakari Mrutu. Their short-term training was on molecular diagnostics of cassava viral diseases at TARI–Mikocheni Biotechology Laboratory. This contributed to their research on the validation of the TAS-ELISA assay for the detection and discrimination of CBSVs in cassava planting materials.

Mr Lwabulala's research validated the efficacy of MAbs for detection of CBSVs infecting cassava. The validation of the ELISA kit involved determination of the sensitivity, specificity, best plant leaf sampling position/zone and the cost-effectiveness compared with gold standard RT-PCR (Mbanzibwa et al., 2011) for routine detection of CBSVs in cassava planting materials. TAS-ELISA was evaluated using five sets of antibodies: two MAbs each for detection of CBSV and UCBSV and one combination of antibodies (Lwabulala, 2018) to simultaneously detect both CBSV and CBSV that was procured from DSMZ, Germany. The validation was achieved using screenhouse plants of known CBSV status and validated with field samples.

Results showed that RT-PCR was more sensitive (100%) in detecting CBSVs in diluted samples from 1:20 to $1:10^{-4}$ w/v compared to TAS-ELISA MAbs, with 52.5% for CBSV and 72.7% for UCBSV. The combined antibodies had specificity of 100% for both CBSVs. The CBSVs were more readily detected in lower than upper leaves, with positive detection of 46.7% and 64.7% for CBSV- and UCBSV-MAbs, respectively. Detection was higher in co-infected than in singly infected samples.

The RT-PCR technique (Mbanzibwa et al., 2011) was more specific (100%) compared to TAS-ELISA with 60.8% and 59.09% true positive detection for CBSV and UCBSV, respectively. Although TAS-ELISA takes more time than RT-PCR to analyze 100 samples (an extra 10 hours and 30 minutes) it is

still cheaper and has a better cost-effectiveness ratio of effectiveness for RT-PCR and analyzes 24 samples more than RT-PCR (Lwabulala, 2018, MSc Thesis, available on the CDP intranet on the Agshare.Today platform).

The validation study concluded that although efficacy of RT-PCR in detection and discrimination of CBSVs was twice that of TAS-ELISA, its practicability in certification schemes where large numbers of samples must be screened in a short time needs to be evaluated. Factors to take into account are the cost of reagents and the technical know-how in operation and equipment compared to those associated with TAS-ELISA. This work enabled us to recommend that TAS-ELISA be combined with RT-PCR to lower the cost of testing large numbers of samples.

Strengthen capacity for seed certification

Among the activities planned by the CDP in supporting seed certification was to strengthen capacity for seed certification. TARI–Mikocheni, in collaboration with the International Institute for Tropical Agriculture (IITA), through the 5CP project have supported TOSCI in developing root and tuber crop laboratory standards and certification guidelines. Both TARI–Mikocheni and IITA through technical meetings (minutes available on the CDP intranet on the Agshare.Today platform) and tailor-made training events on seed inspection and testing supported TOSCI in laboratory testing level, methods of testing, scheme for field testing and determination of prices for testing.

The support enabled availability of certification guidelines for root and tuber crops including cassava, sweet potato and potato. The certification guidelines are now incorporated into the Seed (Amendment) Regulations 2017 and are in operation since its announcement in Government Notice No. 6 in 2017. The guidelines stipulated specific procedures for the production and certification of four classes of seed: pre-basic, basic, certified1 and certified2. The guidelines also provide information on how to obtain certified materials.

Field-based diagnostic kits supplied to TOSCI

In an attempt to support certification system in Tanzania, in addition to building capacity of TOSCI in human resources, TARI–Mikocheni planned to supply TOSCI with field-based diagnostic kits such as ELISA kits, degenerate primers and Loop mediated isothermal amplification (LAMP) to enable them to test for cassava viruses in seed certification labs. In 2014, using CBSV sequences obtained from the 2013 country-wide surveys, TARI–Mikocheni developed four pairs of polyclonal antipeptide antibodies (Table 2) in order to develop a field-based diagnostic kit for CBSVs to supply TOSCI for rapid screening of planting materials.

The purified rabbit polyclonal antibodies (IgG) were evaluated for their detection sensitivity using different assays including nitrocellulose membrane (NCM), antigen coating plate (ACP) and TAS-ELISA and specificity with western blot and immunosorbent electron microscopy. For the direct ELISAs and NCM- and ACP-ELISAs, TARI–Mikocheni's IgGs were used as detecting antibodies (1:250). The rest of the procedures for NCM-, ACP- and TAS-ELISA were performed as described in the International Center for Potato Research (CIP) NCM-Kit and DSMZ protocols, respectively.

Table 2 Types of antibodies and plant materials used for evaluation of IgGs sensitivity by ELISA

SN	TARI–Mikocheni antibodies	Virus spp. target	Plant sample	Plant spp.	Virus status
1	EB-139	CBSV	Tan70	NB	CBSV
2	EB-140	CBSV	Tan70	Cassava	CBSV
3	EB-142	CBSV	Ke125	NB	UCBSV
4	EB-145	CBSV	Ke125	Cassava	UCBSV
5	EB-146	UCBSV	Health	NB	Health
6	EB-147	UCBSV	Health	Cassava	Health
7	EB-148	UCBSV			
	DSMZ antibodies	NA	NA	NA	NA
1	IgG-949/1	NA	NA	NA	NA
2	IgG912/1	NA	NA	NA	NA

Results showed that the specificity of the purified TARI–Mikocheni IgGs at the dilution of 1:500 was low and two of the IgGs, EB-139 and 140, specifically reacted with a 45-kDa protein from total proteins extracted from sample Tan70 pre-inoculated with a CBSV isolate. The IgG EB-146 also specifically reacted with a 43-kDa protein from total proteins extracted from sample Ke125 pre-inoculated with an UCBSV isolate. However, two IgGs EB 142 and 145 raised against UCBSV CP did not react to any total protein – and IgG EB147 also raised against UCBSV CP cross-reacted with total proteins of 45 kDa from total protein extracted from samples Tan70 and Mo22**,** both pre-inoculated with CBSV isolates.

The overall signals were very low, indicating low sensitivity, and IgG EB147 lacked specificity in differentiating CBSV species. These results demonstrated that in the case of the purified antipeptide IgGs raised against CBSVs CP, few were specific to their native CP and their sensitivity was extremely low.

The sensitivity evaluation indicated that all seven IgGs from TARI–Mikocheni (Table 2) had extremely low sensitivity and some did not react at all to their native CBSV CP. In all assays, the highest positive value obtained in all combinations was 1.2 OD_{405nm} and this was only from NB after incubation at 37˚C for 90 minutes. None of the cassava samples had a positive reaction.

Whether TARI–Mikocheni's purified antipeptide IgGs would decorate virions was investigated using DSMZ IgG antisera for coating Ni grids (15') followed by adsorption of sap from virus-infected plants (o/n) and by floating the grids on droplets of antipeptide IgGs for decoration. The immunosorbent electron microscopy results indicated that the IgGs EB139, EB140, EB142 and EB145 showed weak decoration of virus particles; however, in all other EB preparations, virus particles were trapped but not decorated. The weak response of the antisera indicated that peptide antisera were specifically decorating virus particles in EM but nevertheless the reaction was too weak for other tests such as ELISA.

The efficacy of the seven antipetide IgGs evaluated in 2014 and 2015 showed that they were extremely low in specificity and sensitivity in detecting the native virions (CBSVs) raised against it. As certain IgGs cross-react with plant proteins, further validation was required to ensure that their

efficacy reached appreciable specificity and sensitivity. Fresh antibodies were delivered for TARI–Mikocheni validation testing before producing field-based kits to share with TOSCI.

Reaching farmers directly and through partners

Farmers trained on CMD and CBSD disease symptom recognition and management strategies

Among the CDP strategies employed to manage CMD and CBSD were awareness creation on cassava viral diseases and their integrated disease management by demonstrating the benefit of using clean virus-free planting materials. Starting in April 2014, this project conducted outreach programs to reach farmers directly and through various stakeholders. It mainly focused on four districts in three agro-ecological zones: Butiama and Rorya in Mara region in the Lake Victoria basin, Mkinga in Tanga region in Eastern zone and Mbinga district in Ruvuma region in the southern highlands zone.

The four districts were those most affected by CBSD to the extent that farmers were abandoning cassava cultivation. Many farmers were faced with a food deficit due to a shortage of cassava planting materials. Therefore, following discussion with the Ministry of Agriculture, four districts were included in the short-term plan for government intervention to mitigate the CBSD problem.

In collaboration with local governments of the respective districts, TARI–Mikocheni implemented the following steps. Firstly, we created awareness campaigns on the continued threats posed by CMD and CBSD through training of extension agents, crop inspectors, local authorities, decision makers and farmers. Secondly, we provided enhanced solutions through production and dissemination of accessible information to stakeholders and farmers through posters and leaflets in local languages, radio and TV programs, and farmer field days, Thirdly, we set up farm demonstration fields to demonstrate the benefit of using virus-free planting materials to farmers; and, lastly, we disseminated virus-free planting materials to farmers.

When creating awareness of cassava stakeholders (smallholder farmers and extension agents), the approach used entailed the use of both agronomic practices required for cassava production and knowledge on the epidemiology of the two diseases such as causative agents, recognition of symptoms and their management strategies. The training campaigns were conducted in all four districts each year. Both theoretical and practical training were conducted in fields to selected farmer groups. The groups comprised a minimum of 20 farmers, together with their extension agents. Altogether six farmer training programs were conducted in which a total of 987 farmers and 144 extension agents were trained on various aspects of cassava viral disease epidemiology and management.

Additionally, a total of 556 farmers from four districts attended farmer field days in Butiama and Rorya in 2015, and Ilelea village in Mbinga district in 2016. During these field days, farmers played a leading role in sharing the knowledge of cassava viral disease management and in showcasing their demonstration field to the general public.

Demonstration plots for benefits of using virus-indexed planting materials established on-farm

The project established a total 67 demonstration fields in four districts in order to enable farmers to appreciate the benefit of using virus-free cassava planting materials. The establishment of demonstration fields was voluntary through farmer groups, which comprised a minimum of 10 farmers. The establishment was preceded with training both on good agronomic practice for cassava and integrated disease management of cassava viral diseases.

The format of demonstration fields (Figure 17) was set to achieve two important objectives: to compare farmers' preferred planting materials to improved materials in a 10 m × 10 m subplot and the rest of the plot was used to train farmers on how to multiply their own virus-free planting materials using initial stalks of virus-tested planting materials from research institutes supplied by CDP.

Figure 17 (left) Farmers setting up a demonstration plot; (right) Farmer group members showing comparison between improved Mkombozi variety and their local landraces

By using their preferred materials in parallel with improved virus-tested planting materials, the farmers could see the differences between the two sets of plants. The training in the demonstration fields enhanced the farmers' knowledge base on cassava cultivation and integrated management of cassava viral diseases.

Information materials developed and disseminated

Training

The knowledge of cassava stakeholders was enhanced through various strategies. In three years, the project developed its communication strategies with specific messages to different audiences and devised suitable communication media to successfully reach out to stakeholders using various training aids. Several communication channels were employed (Table 3).

Dissemination of clean cassava planting materials to farmers

Following training of farmers on how to recognize cassava viral diseases through demonstration fields, TARI–Mikocheni coordinated the dissemination of virus-tested planting materials from reliable sources: research institutes and private farmers recognized by the Mennonite Economic Development Associates (MEDA) project. The dissemination of virus-free planting materials in the targeted districts enabled further multiplication and spread of improved cassava planting materials to farmers who previously had no information and access to sources of improved materials. Thus, in the past three years, a total of 837,000 cassava cuttings from three improved varieties

(Mkombozi, Mkuranga 1 and Kiroba), kindly donated by cassava breeder Dr Kiddo Mtunda, were distributed to 67 farmer groups in four districts (Table 3).

Table 3 Communication strategies used to train cassava stakeholders

S/N	Target audience	Objectives	Key message	Channel of communications	Frequency	In charge
1.	Farmers	Create	Awareness of cassava diseases and their management	Flyers, posters, brochures, newspaper, fact sheet, radio and television, meetings, workshops and exhibitions	Various	Team and/or extension service
2.	Government institutions (Plant health services, ext, crop protection, NARS)	Build	Capacity to recognize, score, index and select for disease resistant	Trainings, exhibitions, publication, joint meeting	Weekly, monthly, annually	Team
3.	Scientists (plant breeders, plant virologists, entomologists)	Build	Capacity to recognize, score, index and select for disease resistant	Meetings, scientific reports, publication, workshops	Weekly, monthly, annually	Project management
6.	Public	Create	Awareness of the project output	Posters, flyers, fact sheet, newspaper, newsletter, exhibition, radio, television, website/blog	Various	Project team

A total of 10,000 leaflets and over 5,000 posters (Figure 18) on cassava viral disease symptoms and management were developed, packaged into local languages and disseminated to stakeholders – farmers, extension agents and district leaders – to create awareness on cassava viral diseases. Government institutions and scientific communities related to agriculture were periodically reached through 14 public seminars. The general public was well reached through public media including newspapers and more than 20 radio programs both on major and community village radio. In addition, they were reached through TV programs specifically on cassava disease management. In four national annual agricultural events, TARI–Mikocheni reached more than 500 farmers who visited the TARI–Mikocheni stall under the Director of Research and Development of the Ministry of Agriculture Pavilion during shows.

Figure 18 Cassava viral disease training manuals and leaflets developed and disseminated to farmers during awareness campaign creation training

Through CDP's collaboration with the WAVE project, West African farmers in Kebbi State in Nigeria were also reached by exchanging CDP leaflets translated into Hausa and shared with the Cassava Viruses Project in Northern Nigeria (PEARL Project) led by Dr Ibrahim Mohammed.

Build sustainable regional capacity

Strengthening stakeholder linkages

In Phase I, a shared vision and workplan was established among the project countries and cassava stakeholders. Enhanced human capacity and basic infrastructure were built into national programs in all seven partner countries. *Ad hoc* advocacy for enabling biotechnology policies in Tanzania was conducted through radio, TV programs, participation in open fora with the public, interviews with newspaper journalists and support to the Ministry of Agriculture in preparing Cabinet papers.

Project inception and consultative meeting with stakeholders

The Regional cassava viral disease diagnostic project inception meeting was conducted during 14–16 February 2013 at the White Sand Hotel, Dar es Salaam, and attended by 70 participants from different countries especially the project's implementing African countries. As the project executing institute, TARI–Mikocheni continued to conduct monitoring and evaluation visits to its project partners once per year. During those visits, the CDP management team met with the local team and collectively agreed on project objectives and milestones, planned the execution of yearly activities, provided technical backstopping to troubled activities and collected the views of partners on the project output results dissemination plans.

The monitoring and evaluation meetings conducted during the project implementation period enabled the successful recruitment of six MSc and three PhD students from partner countries, informed different cassava stakeholders in the project countries about the project and defined the roles of different stakeholders in assisting the project. This approach significantly helped to build a sense of project ownership in the respective countries.

Stakeholder engagement

As a way of building sustainable national capacity in disease diagnostics, TARI–Mikocheni strengthened its linkages with stakeholders by enhancing the accessibility of information and technologies to farmers through stakeholders. Thus, TARI–Mikocheni worked with several stakeholders to enhance accessibility of information to farmers (Table 4).

Table 4 Name and institutions that collaborated with CDP

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
Sugar Research Institute (SRI)-Root and Tuber Programme	Kibaha	NARS cassava breeder	Stanslous Tolano (Researcher), Caroline Sichalwe (Researcher)	Breeding	Dr Kido Mtunda
		BMGF supported project on cassava community phytosanitation	Dr Kido Mtunda (Project Coordinator/ Cassava Breeder)	Manage CBSD through Community Phytosanitation Project	Dr Kido Mtunda
University of Dodoma	Dodoma	University (Stakeholder)	Dr Chrispin Rubanza (Head of Conservational Biology, Faculty of Biological Sciences)	Training and supervision of students	Dr Chrispin Rubanza
International Institute of Tropical Agriculture (IITA), Tanzania	Mikocheni, Dar es Salaam	BMGF supported project on cassava breeding-New Cassava Varieties and Clean Seed to Combat CBSD and CMD (5CP)	Dr Edward Kanju (Project Coordinator & Cassava Breeder)	Cassava breeding/ improvement	Dr Edward Kanju
		International collaborator on cassava research	Dr James P. Legg (Plant Virologist), Mr Rudolf Shirima (Research Assistant)	Cassava virus research on 5CP and community phytosanitation	Dr James P. Legg
Sokoine University of Agriculture	Morogoro	University (Collaborator)	Prof Cornel Rweyemamu (Head of Faculty of Crop Sciences)	Training and supervision of students	Prof Cornel Rweyemamu
University of Dar es Salaam (UD)	Dar es Salaam	University (Collaborator)	Dr Glady Elibariki	Training and supervision of students at the Department of Applied Microbiology	Dr Glady Elibariki

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
Agro Biotech and Agri Products Ltd	Dar es Salaam	Private tissue culture laboratory	Mr Prashani Patel (Chairman)	Production of disease-free planting material using tissue culture	Mr Prashani Patel
Ministry of Agriculture, Plant Health Services, Crop Department	Dar es Salaam	Government	Ms Dorah Amuli (Plant Health Officer)	Seed Certification	Ms Dorah Amuli
Tanzania Official Seed Certification Institute (TOSCI)	Morogoro	Government	Ms Tasiana Maingu (Acting Chief Seed Certification Officer)	Seed certification	Ms Tasiana Maingu
Upendo Women Farmer Cooperative (Mbinga)	Mbinga	Farmers	Several farmers	Cassava production, hosting and maintaining demonstration plot and cassava multiplication	Group leader
Ukiriguru Agricultural Research Institute (UARI)	Ukiriguru, Mwanza	NARS	Dr Simon Jeremiah Satu (Principal Research Officer)	Research and improvement of cassava	Dr Simon J. Satu
Crop Biosciences Solutions Ltd	Arusha	Private tissue culture laboratory	Mr Wilfred Mushobozi (Chief Executive Officer)	Production of disease-free planting material using tissue culture	Mr Wilfred Mushobozi
The Nelson Mandela African Institution of Science and Technology (NM-AIST)	Arusha	University	Prof Patrick Alois Ndakidemi (Deputy Vice Chancellor/Senior Lecturer in Sustainable Agriculture)	Training and supervision of students	Prof Patrick A. Ndakidemi
Zanzibar Agricultural Research Institute (Zanzibar ARI)	Zanzibar	NARS	Mr Haji Saleh (Officer In Charge), Mr Shaali Mohamed Shaali (Research Assistant)	Research and improvement of cassava	Mr Haji Saleh

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
District Agricultural Offices	Butiama-Mara, Rorya-Mara, Mkinga-Tanga, Handeni-Tanga, Kisarawe-Coast, Mbinga-Ruvuma	Agricultural extension services	District Agricultural Officers and extension agents	Farmer training, dissemination of IPM packages and virus-free planting materials	District Agricultural Officers and extension agents

Through engagement with various stakeholders, TARI–Mikocheni's capacity for virus diagnostic and dissemination of disease integrated packages became more accessible in the country. A significant number of postgraduate students and research assistants were trained. Additionally, the availability of virus-free cassava planting materials was enhanced and integrated pest management packages were simplified through farmer groups.

Exchange visits between scientists in the project countries

At the beginning of the project, it was realized that the participating countries were at different levels in terms of human capacity, scientific experience, infrastructure and biotechnology. Thus, one strategy to achieve the project goals was to share experience through exchange visits between partner countries. During 16–20 May 2016, three CDP scientists/project coordinator and project accountants participated in the first CDP exchange visit hosted by the Zambia Agriculture Research Institute (ZARI), a sub grantee and CDP partner in Zambia. During the visits, the CDP team comprising country team leaders from Kenya, Rwanda, Uganda, Malawi and Mozambique visited fields of farmers collaborating with the project and noted the benefit of using clean materials. Additional locations visited included the diagnostic laboratory at ZARI and enabled the CDP visitors to exchange experiences on project activities.

Outreach to regional virologists in non-project countries

One of the key aims of CDP was to build sustainable regional capacity in cassava viral disease diagnosis and management among the partner countries and share the outputs with non-project countries facing similar challenges in cassava production. In Phase I, the project concentrated on enhancing capacity mainly in the key project countries of Kenya, Malawi, Mozambique, Rwanda, Uganda, Tanzania and Zambia. The success of Phase I generated huge interest in the scientific community working on cassava viruses in other African countries. Thus, in Phase II, CDP scaled up its efforts to share project outputs and experience in disease diagnostics to scientists outside its key project countries, and included Madagascar, Democratic Republic of Congo, Congo, Burundi and West Africa.

During 16–19 June 2015, three TARI–Mikocheni-based scientists participated in the launching of West African Virus Epidemiology (WAVE) for Root and Tuber Crops program in Abidjan, Côte D'Ivoire. Along with the launching of the program, CDP scientists also provided on-site training on

conducting disease surveys, symptom severity scoring, whitefly counting and collection, and sample collection to the WAVE team. The CDP scientists also participated in WAVE annual meetings in Abidjan, Côte D'Ivoire in 2016, and Kebbi State University and Abuja in Nigeria on October 2017 (presentations available on the CDP intranet on the Agshare.Today platform). Following these visits, the collaboration between WAVE and CDP grew, and in June 2017 one TARI–Mikocheni scientist, Ms Leonia Mlaki (Figure 19), was invited to provide technical support on whitefly biotyping and molecular characterization to WAVE scientists at the WAVE biotech laboratory in Abidjan, Côte D'Ivoire.

Figure 19 TARI–Mikocheni scientist Ms Leonia Mlaki in the laboratory (at microscope and pipetting) providing technical backstopping on biotyping using microscope and molecular characterization using PCR with *mt-CO1* primers to WAVE scientists at WAVE biotech laboratory in Abidjan, Côte d'Ivoire in July 2017

During 4–11 January 2017, a team of five scientists led by Dr Joseph Ndunguru and including Dr Fred Tairo, Dr Peter Sseruwagi, Dr Laura Boykin and Ms Jeanine Umfuyisoni visited the Madagascar Agricultural Research Institute (FOFIFA) to establish collaboration with counterparts on cassava viral disease research and management. The team met with the scientists, ministry and private sector people involved in cassava processing.

In Madagascar, the team met the Assistant Director of FOFIFA and the Director General of Agriculture at the Ministry. Among the topics discussed were possible areas of collaboration and expertise exchange. One identified area of interest was capacity-building in cassava viral diseases, vector surveillance and mapping, infrastructure and human resources. The two teams agreed to carry out a joint country-wide survey for cassava viral diseases with a Madagascar team as a continuation of the capacity-building strategy.

In addition, the CDP team visited 14 cassava farmers' fields in 13 locations within two main cassava agro-ecological zones west of Antananarivo and in the Mahajanga region, northwestern Madagascar. The field visits and visual cassava disease assessments, whitefly counts and non-cassava plants with virus-like symptom assessment showed that most were infected with CMD. The incidence of CMD ranged within 30–100%; cuttings were the main source of disease infection, as indicated by the nature of symptoms (Madagascar report available on the CDP intranet on the Agshare.Today platform).

During the eight-day visit to Madagascar the CDP team and the Madagascar cassava team found that both countries shared similar challenges concerning cassava viral diseases, particularly CMD and probably CBSD and whitefly. The common challenges can be addressed efficiently by working together and using a common approach. Despite differences in capacities between East Africa and Madagascar, both countries can benefit through collaboration and exchange of expertise in various

aspects of cassava research. The CDP team and the Madagascar team, both at Ministry and FOFIFA levels, agreed to collaborate on cassava research, thus producing a rapid build-up of capacity for addressing cassava viral diseases.

Strengthening human capacity and infrastructure

Human capacity

Prior to CDP, there was very low human resources capacity in Tanzania with the technical expertise to carry out disease surveillance of cassava viral diseases, monitoring and certification of cassava planting materials. Thus, one of the CDP's key priorities was the recruitment and training in long- and short-term training courses of human resources to ensure implementation of CDP goals. During 2009–2017, the CDP invested significantly in enhancing human resources in both technical and supporting capacities at TARI–Mikocheni and its national partners with the recruitment of at least 23 staff on the project (Table 5).

Table 5 Staff recruited at TARI–Mikocheni, 2009–2017

No.	Name	Position
1	Joseph Ndunguru	Project Coordinator
2	Fred Tairo	Assistant Project Coordinator – Outreach & Training
3	Peter Sseruwagi	Assistant Project Coordinator – Technical
4	Shubila Katagira (RIP)	Project Accountant
5	Cecilia Sunga	Administrative Assistant
6	Charles Kayuki	Lab Manager
7	Hilda Bachwenkiz	Research Assistant
8	Ramadhan Lipala	Research Assistant
9	Laurencia Mushi	Research Assistant
10	Rahma Mkangwa	Research Assistant
11	Tursiuis Fute	Technician
12	Deogratius Mark	Technician
13	Joel Erasto	Technician
14	Shamsa Kileo	Technician
15	Veneranda Mlegi	Technician
16	Margareth Lupembe	MSc student
17	Veneranda Ngazi	MSc student
18	Maliha Saggaf	MSc student
19	Christina Kidulile	MSc student
20	Dickson Lwabulala	MSc student
21	Cyprian Rajabu	PhD student
22	Mapambano Kisendi	Driver
23	Honest Kway	Driver

Long-term training

MSc

Of the 14 MSc students directly supported by the CDP, four were recruited in Phase I and 10 in Phase II, and TARI–Mikocheni trained seven students. In addition, to CDP-direct support, TARI–Mikocheni also partially supported five students with funds to undertake research on various topics of disease diagnostics on the three project aims described in Section Three. The areas of research of the students are presented in Table 6, showing student training and research areas for Phases I and II. The MSc students were sourced among TARI–Mikocheni staff, TOSCI and private tissue culture laboratories involved in multiplication of planting materials. The successful completion of their studies significantly enhanced the capacity of TARI–Mikocheni and its key stakeholders; it created a critical mass of trained young scientists in disease diagnostics in Tanzania. One useful output generated by MSc researchers was in the area of cassava breeding. More specifically, this covered the nature and effect of interaction between CMBs and/or CBSVs with eSEG-S and the mechanism of resistance. The information generated was published in several journals and theses (see References).

PhD

Following the successful completion of Phase I training strategies at MSc level, TARI–Mikocheni continued to build up its critical mass of expertise in virology by training one PhD student Mr Cyprian Rajabu, who was a beneficiary of the Phase I MSc program. Mr Cyprian Rajabu was among five PhD students recruited in Phase II. He was enrolled at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya although he did all his research work at NCSU, USA, under supervision of Professor Linda Hanley-Bowdoin. His research on the characterization of cassava begomovirus infection in cassava – in the presence or absence of sequences enhancing geminivirus symptoms – generated very useful and publishable information on the infection caused by geminiviruses in model host plants (Rajabu et al., 2018) and the mechanism employed by SEGS-1 to break host resistance in CMD-tolerant cassava (Ndunguru et al., 2016). The findings of his research shed significant light on the roles of SEGS-1 in cassava mosaic epidemics and gave insight on how to better manage the disease through breeding of cassava varieties with durable resistance against CMBs of the *Geminiviridae* family.

Short-term training

The capacity of the project scientists, research assistants and the supporting staff of TARI–Mikocheni was well enhanced through retooling training in diagnostics and various professional expertise (Table 7). Three project scientists and coordinators, 39 research assistants and six supporting staff of both CDP and TARI–Mikocheni received advanced professional training and refreshed them with advanced skills to enhance their efficiency. The training scheduled was short-term (5–30 days) within the region and overseas for specific skills for which capacity was not available in the region. They were mostly conducted in-house and through short attachments (30 days) to advanced laboratories in Julius Kuhn Institute, Germany, the Energy and Biochemistry Department of the University of Western Australia (UWA) and at the Department of Ecology, Evolution and Natural Resources, Rutgers University, New Brunswick, NJ, USA.

Table 6 Summary of TARI–Mikocheni postgraduate students trained during 2009–2017

	Student name	Program	University	Year	*Research topic	Product
1	Cyprian Rajabu	MSc/PhD	University of the Witwatersrand, South Africa (Wits-SA)/ JKUAT-KE	2012	Characterization of cassava begomoviruses infection in cassava in the presence or absence of sequences enhancing geminiviruses symptoms	Developed m-PCR primers for simultaneous detection of CMBs in cassava
2	Habibu Mugerwa	MSc/PhD	Wits-SA/NRI	2012	Molecular variability of cassava *Bemisia tabaci* and its effects on the spread of CMBs in East Africa	Dynamics and biology of virus vector *Bemisia tabaci* spread CMD in the region elucidated
3	Happiness Gabriel	MSc/PhD	Wits-SA/UMA-SP	2012	Interaction and impact of CMBs and their associated satellites	Information on nature of interaction and impact of the resistant-breaking DNA satellite on cassava in association with CMBs
4	Catherine Gwandu	MSc	SUA-TZ	2014	Epidemiological aspects of CBSD in field grown cassava in coastal regions of Tanzania	Epidemiology of CBSD elucidated on nature of spread within cassava fields
5	Margreth Lupembe	MSc	SUA-TZ	2017	Molecular diversity of DNA-B components of East African cassava mosaic viruses in Tanzania	Resolution of molecular diversity of CMBs in Tanzanian refined
6	Christina Kidulile	MSc	SUA-TZ	2018	Tissue culture and transformation strategies for low-cost delivery to farmers	
7	Veneranda Ngazi	MSc	MAK-UG	2018	Evaluation of cassava genotypes for resistance to CBSD in Tanzania-	Nature of resistance of cassava against CBSVs infection
8	Dickson Lwabulala	MSc	UoN-KE	2018	Evaluation of efficacy of ELISA for routine indexing of CBSVs in cassava planting materials	Validated TAS-ELISA tool for detection of CBSVs

	Student name	Program	University	Year	*Research topic	Product
9	Maliha Saggaf	MSc	UoN-KE	2018	Determining the possible interaction of sequence enhancing geminiviruses symptoms with CBSVs	Synergist effect of eSEGS1 and eSEGS2 in co-infection with CBSV in cassava elucidated
	Ramadhani Makaranga	MSc	SUA-TZ		Survey for natural eukaryotic translation initiation factor variants in cassava for identification of sources of cassava brown streak potyvirus resistance	Diversity of translation initiation factor (eIF4e and elf4G) in CBSD-resistant and -susceptible varieties identified as potential candidate for resistance
10	William Moshy	MSc	SUA-TZ	2016	Association of *BADH2.1* gene allele with aroma in popular traditional rice variety	Identified genes for aroma in rice varieties
11	Amar Mussaji	MSc	Nottingham, UK		Tissue culture strategies for low-cost delivery to farmers	Tissue culture protocols for planting materials
12	Olga Naomi Kamanga	MSc	SUA-TZ	2016	Determination of the level of expression of *OSCIPK15* salt response gene in selected Tanzanian rice landraces	22 rice lines with high level salt response gene (*OSCIPK15*) identified
13	John Soleemulo Fayiah	MSc	SUA-TZ	2016	Survey for the expression levels of drought tolerant genes in cassava varieties in Tanzania	Eight drought-tolerant cassava varieties identified and their expression quantified with upregulated genes (ALDH7B4, ZFP252, MSD and RD28)
12	Joel Erasto	Diploma	PolyTech.KE	2016		Diploma in Lab. Technology

***Copies of MSc theses are available at TARI–Mikocheni for reference**

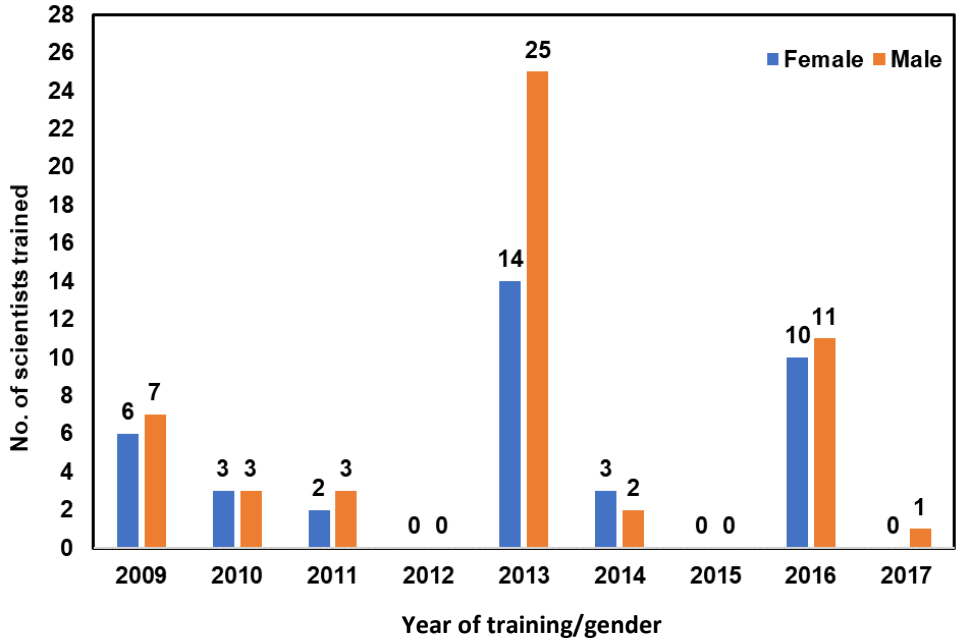

Figure 20 Summary of TARI–Mikocheni scientists (by gender) trained during 2009–2017

Table 7 Summary of short-term training for capacity-building of TARI–Mikocheni staff during 2009–2017

Professional training	Year	Venue
Virus diagnostics and laboratory practices	2009/2013	TARI–Mikocheni Biotech Lab-DSM, Tanzania
Scientific writing skills	2010/2016	Mombasa, Kenya; San Diego, USA and Lusaka, Zambia
Basic laboratory safety training	2011	BecA-ILRI, Nairobi, Kenya
Procurement skills	2012	Dodoma, Tanzania
Leadership skills	2013/2014/2017	Entebbe, Uganda; Kigali, Rwanda and London, UK
Intellectual property and communication training	2014	ICRAF, Nairobi, Kenya
Accounting and financial management – Mango	2013/2016	DSM, Tanzania
Data management and sharing skills	2015	DSM, Tanzania
HFP women leadership training	2016	San Diego-USA
Advance protein-based diagnostics tools	2016	Braunschweig, Germany
Next-generation sequencing	2016	UWA
Bioinformatics training	2016	TARI–Mikocheni Biotech Lab-DSM, Tanzania

Infrastructure enhancement

Over the past 8 years, CDP contributed to the infrastructure at TARI–Mikocheni, enabling the institution to conduct quality research, in parallel with enhancing human-resources research capacity. Some US$780,000 was invested in infrastructure; TARI–Mikocheni upgraded its three laboratories with both the basic and advanced equipment necessary for a variety of diagnostic and molecular biology related work (Table 8).

Table 8 List of laboratory equipment at TARI–Mikocheni Biotech Laboratories procured by the CDP and their working condition

SN	Equipment	Serial Number	Function	Qty	Status
1	Real-time PCR (MX300P)	DE92800335	Quantitative PCR analysis	1	Good working order
2	Autoclave	0.20488	Autoclaving	1	Good working order
3	Growth chamber	W090130	Controlled growth		Brand-new-needs fixing
4	Water purifier	32950	Distilled water	1	Good working order
5	Ultralow freezer-80C	03.0348	Sample preservation		Good working order
6	Refrigerated microcentrifuge	157872 157870	Sample preparation	2	Good working order
7	Microcentrifuge	5418Y0413032 5418Y0713029	Sample preparation	2	Good working order
8	Laminar flow air cabinet	Not available	Tissue culture isolations	2	Good working order working
9	Ice maker	DD7666101	Ice making	1	Good working order
10	Dissecting microscope	DM143 series	Magnification	1	Good working order
11	Nanodrop 2000 spectrophotometer	C553	Nucleic acid quantification	1	Faulty-requires authorized repair
12	Geno grinder 2010	10246	Bulk sample homogenization	1	Good working order
13	Small autoclave	DA039Y	Sterilization	1	Good working order
14	Large autoclave	2993	Sterilization	1	Good working order
15	Water bath	162623-15	Heating samples	1	Good working order
16	Freezer (–20°C)	8037509	Sample preservation	1	Good working order
17	Refrigerator (+4°C)	8037507	Sample preservation	1	Good working order
18	pH meter	HI 5221	Buffer preparation	1	Good working order
19	Freezer (BIOCOLD)	46030082	Sample preparation	1	Good working order
20	Refrigerated microcentrifuge	0000665-01-00	Sample preparation	1	Not working
21	Water distiller		Water purification	2	1. Good working order 2. Needs repair
22	Water distiller	F1E090016 F1K030014	Water purification	2	Not working Needs repair
23	Water purification syst.	FOJA53497	Water purification	1	Good working order
24	Rotor Unimax type Multi reax	061006022 071001419	Mixing of sample content	2	Good working order
25	Heating block r	R000101635 R000104746 R000104761	Heating of samples	3	Good working order
26	Laminar flow cabinet	Unavailable	Sterile work space		Good working order
27	Microwave oven	81000900361410 287708	Heating of media		Good working order
28	Water bath 20 L	115926 115927	Heating of samples/buffers	2	Good working order

In addition to equipment, CDP also complemented government efforts to provide TARI–Mikocheni with reliable transport to conduct research activities. CDP facilitated the procurement of two four-

wheel drive all-terrain vehicles (Table 9), which were essential for country-wide surveys and transport within the region.

Table 9 List of vehicles procured by TARI–Mikocheni in 2009 and 2013 for CDP activities

SN	Name of equipment	Chassis number	Function	Year	Status
1	Land Cruiser-Hardtop	TERB715300051408	Transport	2009	Good working order
2	Land Cruiser Prado	JTEBD9FJ30KO127572986ML	Transport	2013	Good working order

Similarly, TARI–Mikocheni's research facilities were enhanced by the refurbishment of two screenhouses. A further outbuilding was refurbished and equipped to provide a virus-indexing laboratory to support TOSCI and other stakeholders for certification of planting materials.

A further upgrade of TARI–Mikocheni's facilities was to enhance its research capability and efficiency by connection to the fiber optic internet, which boosted its efficiency in communicating with its collaborators. Following bioinformatic training conducted by our collaborators from UWA, TARI–Mikocheni procured bioinformatic hardware and software (Table 10) and connected to the supercomputer based at UWA. This greatly enabled TARI–Mikocheni scientists and postgraduate students to complete their analyses utilizing the facility and collaboration through Dr Laura Boykin, UWA. Furthermore, with the assistance of AgShare.Today, TARI–Mikocheni designed and established an institute website with a site for CDP.

Table 10 Research facilities procured at TARI–Mikocheni by CDP project between 2009 and 2017

SN	Name of facility	Function	Qty	Status
1	Mac Pro computer, 8 TB backup	Analysis	1	Good working order
2	CLC and Geneious software licenses	Analysis	2	Up to date
3	Internet-fiber (10 MBPs)	Communication	1	Good working speed
4	Institute website	Communication to stakeholders		In progress

In line with TARI–Mikocheni laboratory upgrades, using its institutional support budget, the three project offices at TARI–Mikocheni were repaired and furnished, and the institute repaired and repainted along with one of its two substations – Mkuranga substation in Coast region.

Thus, with the significant investment the capacity of TARI–Mikocheni to conduct quality research and support other projects and its stakeholders was enhanced, which enabled it to attract more projects. A number of universities using the TARI–Mikocheni Biotech Laboratory has increased from two in 2009 to seven in 2017 (see Section Three).

SECTION THREE: Impacts, success stories and learning outcomes

Impacts

Impact area	Impact
No. of students trained by this project directly and indirectly	TARI–Mikocheni trained 16 postgraduate students; of these nine were fully sponsored and seven partially supported by the CDP. Some of these students are currently working for the government at various research institutions/universities and at non-research institutions contributing to the human capacity strengthening in the project countries.
No. of projects using the CDP facilities	TARI–Mikocheni's facilities, which were enhanced through CDP, are being used actively by 14 projects including PEARL projects (common beans and nematodes), Bio innovate, CMD-FAO, ACWP, IPM-Vegetable, 5CP and GLCI, BREAD, PEER, BecA-Aflatoxin, the International Center for Genetic Engineering and Biotechnology (ICGEB) and Potato-CIP.
No. of students and/or staff using facilities and reagents of CDP	Eight non-CDP postgraduate students used CDP facilities in their studies during the period 2013–2017: **MSc research students** 1. Shedrack Kitimu (ICGEB), 2. Bakari Mrutu (TOSCI) 3. Veneranda Mlegi (COSTECH-GOT) **PhD students** 1. Magdalena (ARI-Maruku-COSTECH) 2. Mariam Mtunguja (TARI–Mikocheni-COSTECH) 3. Chuwa-ARI-Colima-EARPP-GOT 4. Glady Elibariki (COSTECH) 5. Olga N. Kamanga (iAGRI-Zambia) 6. John Soleemulo Fayiah (iAGRI-Ghana) In addition to these students, each year some 30 university students come to TARI–Mikocheni to conduct their laboratory practicals using facilities acquired through CDP. Other students come as interns and some are volunteers who wish to acquire skills.
No. of people that have been inspired by the project	CDP has inspired many researchers in Tanzania and beyond in various ways. Before CDP, BMGF did not directly fund any national research programs in Africa – CDP became the first project of this kind. This inspired many African scientists to write grant-winning proposals to BMGF and other donors. For example, in 2013, over 200 PEARL proposals from

	African scientists were submitted to BMGF and 50 of these projects were funded. CDP also influenced scientists in West African countries and this led to the West African Virus Epidemiology (WAVE) project which is currently being implemented in six West African countries using the CDP model.
Institutional visibility – recognition of the institute's capacities	The visibility of the institution's hosting of CDP has become prominent and has led to the acquisition of more laboratory facilities and infrastructure from other sources of funding, as well as new collaborative projects and joint publications. The number of new stakeholders visiting the research institutes hosting CDP has increased during the project implementation. At TARI–Mikocheni, this has resulted in further funding from e.g. USAID, FAO, Department for International Development and the National Science Foundation.
Infrastructural capacity – helping students execute their projects	Following the acquisition of laboratory facilities at TARI–Mikocheni through CDP, Tanzanian university students who in previous years would have gone abroad to conduct their research work are now using the TARI–Mikocheni facilities. This is a good economic result for all concerned. Facilities available to such students include: 1. Well-equipped molecular biology genotyping laboratory 2. Well-functioning tissue culture lab and growth room 3. Well-equipped Biosafety Level 2 laboratory 4. Well-equipped molecular diagnostic lab 5. Two screenhouses for insect-controlled experiments 6. Fast internet facilities – fiber 7. Accessibility to peer-reviewed journal through AGORA and subscriptions to quality scientific journals, e.g. Plant Disease, Phytopathology and Molecular Plant-Microbe Interactions 8. Bioinformatics facility for sequencing analysis.
New stakeholders interacting with the project	Through CDP, TARI–Mikocheni has been interacting with Kilimo Organo Tissue Culture Laboratory, DSM, Tanzania; Bioscience Solution Company, Arusha, Tanzania; and Tanzania District Agricultural Offices: Butiama-Mara, Mbinga-Ruvuma, Mkinga-Tanga, Rorya-Mara, Handeni, Tanga and Kisarawe-Coast.
Service the lab has provided	Using its biotech facility, TARI–Mikocheni has provided biotech services to various stakeholders

	through collaborative projects and on a cost-sharing basis. Services provided during 2013–2017 include: • 500 potato seed *in vitro* plants were virus indexed and multiplied for ARI-Uyole in 2013 • 7000 cassava planting materials were screened for CBSV certification for Community Phytosanitation Project-Kibaha during 2013–2015 • 31 cassava clones of 5CP project screened and multiplied during 2013–2015 • 20 sugarcane germplasm lines introduced from Mauritius by ARI-Kibaha in 2015 screened for viruses • 906 samples of maize and rice fingerprinted for AGRA-Project in 2015 • 2175 samples of maize, beans and rice processed and DNA extracted for Tegemeo Project in 2016 • Two technicians of Kilimo Organo DSM lab received tailor-made training on tissue culture techniques in 2017.
How many farmers have benefited either directly or indirectly	One of the CDP's aims was to provide farmers with virus-free improved planting material. To this end, 67 farmer groups (15–20 members each) received virus-free planting material for multiplication in 1-acre demonstration fields. Thus over 1000 farmers accessed clean planting materials. Also, farmers accessed training materials and training on how to recognize disease symptoms in their fields so that they can practice selection of clean planting materials and avoid spreading disease through planting diseased cassava.
New collaborations/ collaborative projects	Following enhanced capacities in human resource and infrastructure, TARI–Mikocheni's visibility grew and enabled it to attract collaborative projects. During 2013–2017, TARI–Mikocheni participated in five collaborative projects with specific roles played by TARI–Mikocheni scientists utilizing the biotech facilities available at TARI–Mikocheni. These collaborative projects include: • BREAD project with NCSU during 2011–2013 • PEER project with NCSU during 2012–2014 • TEA project funded by government through COSTECH during 2012–2014 aimed at producing high-quality tea in vitro planting materials for smallholder farmers • FAO-CMD project with JKUAT-Kenya and NEIKER-Spain during 2016–2019

	• CEREAL project funded by BMGF through Ergaton University, Kenya during 2016–2017 with aim of tracking the adoption of improved cereal seed in Tanzania using DNA fingerprinting technology • Bio innovate project funded by SIDA to Makerere University Uganda during 2017–2019, aimed at integrating ICT in commercial production of quality sweet potato planting materials in East Africa • IPM Vegetable – funded by USAID during 2016–2019, aimed at building capacity of vegetable growers in managing virus disease through use of integrated pest management practices in Tanzania • CMD resistance with NCSU during 2016–2020.
People using information generated by this project	Information generated by CDP included disease prevalence maps. Breeders and District Agricultural Officers in the major cassava-growing areas received these maps and these are being used to decide where to deploy, multiply and screen improved materials – as well as to strengthen phytosanitary regulations. Cassava breeders are using virus testing data and germplasm. District Agricultural Officers are using disease maps and virus- tested cassava planting materials.
Benefits to the government -extension training, inspectors and regulators	• 144 extension officers trained in six districts during outreach activities during 2014–2016 • Three crop inspectors from TOSCI trained on field inspection for cassava viral diseases and lab virus detection • 20 technicians from government regulatory agencies – Government Chemist and Laboratories Agency, Tanzania Food and Drug Authority, TOSCI, Tanzania Bureau of Standards and Tanzania Pesticide Research Institute – trained on detection of GMOs in 2012.
Advocacy-impacts on policy etc.	CDP played an advocacy role in Tanzania and this resulted in changes at governmental level on the way business is conducted. For example, through invitation of government officers (His Excellency the President, Ministers, Regional and District Commissioners) to TARI–Mikocheni in recognition of the CDP activities, the project was able to advise the government on a number of issues such as: (1) the review of Tanzanian biosafety regulations that resulted in the removal of a 'strict liability' clause for research, (2) the need to invest more in research in

	order to generate the much needed improved materials required by farmers and (3) the need to engage with the private sector in research for sustainability purposes.
Publications and other communications including other communication materials	• 18 papers published in peer-reviewed journals during 2012–2018 • 10,000 leaflets produced and disseminated during 2014–2017 • Three radio programs produced and aired during 2014–2016 • 11 TV programs produced and aired during 2014–2016 • Six project annual meetings conducted in six project countries during 2010–2017 • 330 project annual proceedings produced and distributed to partners during 2010–2012 • One blog (www.cassavam.blogspot.com) developed and launched during 2013–2015 • One CDP intranet on the Agshare.Today platform (www.Agshare.today/CDP), developed in collaboration with AgShare.Today, launched in 2015 • One TARI–Mikocheni website designed and developed in 2017 (await launching) • Two farmers' field days each in Butiama-Mara and Mbinga-Ruvuma conducted in 2014 and 2017, respectively.
Increase in crop yield and incomes (especially farmers who used clean materials)	Cassava yields in farmer fields planted with virus-free material increased from 5 t/ha (before the project intervention) to 35–45 t/ha. This led to improved household food security and income generated through selling of planting material as well as cassava tubers and chips. Money generated from cassava could be used by farmers to access services such as health and education.
Meetings and conferences attended – for the whole team	One aspect of the knowledge and information sharing adopted by the CDP was its participation in scientific meetings and conferences. For the past four years, both senior scientists and postgraduate students have participated in a number of conferences and presented their work, for example: • Plant and Animal Genome (PAG) conferences in San Diego, USA in 2014, 2015 and 2016 • International Plant Virus Epidemiology (IPVE) conference in Arusha, TZ in 2013 • Global Cassava Partnership (GCP) conference in Kampala, Uganda in 2012

	• Geminivirus conference in Hangzhou, China, in 2013 • International Whitefly Symposium in Arusha, TZ in 2016 • International Society of Root and Tuber Conference in DSM, TZ in 2017 • Grand Challenge Conference in London, UK, in 2017.
Support to breeders and other projects	Following the enhanced capacity of TARI–Mikocheni in biotech infrastructure through CDP, the facility has supported various stakeholders in the country through collaborative projects and service provisions. Since 2012, the main beneficiaries of the support have been breeders of rice, maize and cassava. Some of the support rendered to them includes: • 168 Tanzania rice landraces screened at TARI–Mikocheni using SSR markers for EARP project on 2012 (EARPP report, 2013) • 297 improved maize varieties genotyped and screened for maize lethal necrosis (MLN) at TARI–Mikocheni using SSR markers and MLN component viruses specific primers for WEMA project • 7000 planting materials screened for CMBs and CBSVs for Community Phytosanitation Project during 2013–2016 (Community Phytosanitation Report, 2017) • 509 cassava tissue culture clones from KEPHIS screened for CMBs and CBSVs for 5CP during 2013–2015 • Four improved cassava varieties bred for CBSD resistance by ARI, Kibaha, their CBSV viral load quantified at TARI–Mikocheni (MSc thesis – Ngazi, 2017) during 2013–2016 • 287 cassava cloned tissue culture plants from Natural Resource Institute (NRI), UK, under quarantine at TARI–Mikocheni were screened for CMBs and CBSVs for African Cassava Whitefly Project in 2016.
New businesses initiated as a result of this project	One of the successful established businesses resulting from CDP is the establishment of the private tissue culture laboratory ‘Kilimo Organo’ in 2016 in Dar es Salaam: • The owner Mr Amar Mussaji is one of the beneficiaries of CDP MSc partial sponsorship support. Following the completion of his MSc at the University of Nottingham, UK, he returned

	and established a tissue culture laboratory in 2016 • He continues to receive technical support from TARI–Mikocheni on the virus-indexing of tissue culture materials.
Involvement of vulnerable groups – such as persons unable to give informed consent; children; the elderly; people receiving welfare benefits or social assistance, poor persons, the unemployed, some ethnic minorities, the homeless, nomads, refugees and asylum-seekers, prisoners, patients with incurable disease, politically powerless individuals and members of communities unfamiliar with modern medical concepts	The implementation of CDP took into these criteria into account and involved many of these groups. • Most farmers who received cassava planting materials were women and included elderly people • One cassava demonstration plot was established at Mkuka Primary School in Mbinga district for the benefit of school children, to train them on cassava production and encourage them to disseminate information to their families • Similarly, school children from various secondary schools were invited on excursions to TARI–Mikocheni laboratories to inspire them to study science subjects at school • Young farmers received disease-free planting materials; they grew these to generate income and food.
Change of farmers' perceptions	• Farmers, who either had abandoned growing cassava or were not already growing this crop, started growing cassava as a cash and food security crop using improved materials from CDP. This resulted from seeing the high yields of fellow farmers growing CDP clean materials • Farmers changed their previous perception of cassava as a poor man's crop for food security and began to regard it as a source of income • Previously farmers thought that results from cassava research did not reach farmers and remained in the laboratories. CDP showed that research results can directly benefit smallholder farmers and this led to farmers changing their view of research and researchers.
Other universities requesting to use the facilities/equipment	The infrastructure capacity built by CDP at TARI–Mikocheni has contributed significantly not only to addressing the farmers' cassava virus problems, but has been supporting Tanzania's universities in the field of biotechnology and molecular biology. TARI–Mikocheni has provided access to students for their postgraduate research using laboratory, screenhouse and bioinformatic facilities. For instance, during 2010–2017, seven universities and their students had the opportunity to access biotech facilities at TARI–

	Mikocheni and accomplished research activities: University of Dar es Salaam, Sokoine University of Agriculture, Nelson Mandela University of Science and Technology, Dar es Salaam Technical Institute, University of Dodoma, University of Agriculture Bagamoyo and Mkwawa University.
Minimizing/arresting brain drain	Often after completing their degree training, especially at PhD level, many African scientists seek employment outside Africa because they consider that Africa does not offer the possibility for work and professional skill development. Now, through the regional capacity that CDP has built and strengthened in the project countries, African scientists are motivated to stay in Africa and work on research toward providing practical solutions to smallholder farmers and at the same time advancing their professional skills.
Building a network of scientists	Through CDP we now have a network of scientists in Africa working together with the common goal of addressing cassava and other crop diseases by using standardized and harmonized protocols and procedures. This 10-year-old network (2008–2018) has been very effective and strong in achieving its goals and targets through sharing of results, experiences, knowledge, lessons learned as well as challenges. CDP is now one of the few existing networks in Africa whose success can be a model for other programs to learn from and build on.

Success stories

Through the regional capacity built by CDP in Tanzania, the human and infrastructural capacity for disease diagnostics has been significantly enhanced. Disease diagnostic laboratories are operational and functional. Thus scientists can now go to the field, collect samples and carry out analysis in the laboratories in-country. This has led to mapping of CMD and CBSD in the whole country and the disease prevalence maps are being used to decide on where to deploy, multiply and screen improved cassava germplasm as well as to strengthen phytosanitary regulations. Smallholder farmers received training on CMD and CBSD and use the knowledge in practicing phytosanitation for disease control.

In addition, over 1000 smallholder farmers received disease-free planting materials for further multiplication using farmer groups in six districts: Rorya and Butiama (Mara region), Mbinga district (Ruvuma region), Mkinga and Handeni districts (Tanga region) and Kisarawe district (Coastal region). Farmers who received these virus-free planting materials increased their yields from 5 to 45 t/ha. This resulted in improved household food security and income as well as livelihoods (Figure 21).

Figure 21 (left) Cassava tuber yield from CMD-infected cassava plant; (right) Yield from disease-free plant 10 months after planting in one demonstration field operated by the CDP

Figure 22 PhD and MSc students fully supported by CDP project together with Project Coordinator Dr Joseph Ndunguru (seated second from left)

The project has trained 14 MSc and 6 PhD students as well as several others through short-term support on different aspects of disease diagnostics (Figure 22). This contributed significantly to human capacity-building in the country and beyond and now serves as a very useful resource for training others. The knowledge acquired by these young people is currently being used to enhance research activities in the country and they will be future research leaders in the country.

Learning outcomes

The implementation of the CDP has contributed in changing the way in which researchers interact with cassava stakeholders. Joint meetings between cassava breeders and plant virologists enabled development of common working tools, e.g. a disease scoring manual that helped both breeders and virologists in getting virus disease data. Similarly, CDP enabled virologists to access breeders' varieties during execution of demonstration plots and enabled breeders to access virus data from screening and quantification of virus titers for breeders' materials, which speeds up the release of improved cassava viral disease resistant materials.

Sharing of knowledge, particularly data, was another learning outcome with assistance from AgShare.Today. The adoption of the AgShare.Today platform revolutionized the simplicity of sharing data, protocols and literature within network partners and other networks. Accessibility to data became very easy and fast through an intranet site on the AgShare.Today platform that enabled partners to exchange and access data with each other compared to the previous medium of email.

Procurement of both equipment and consumables has always been a major constraint due to strict and lengthy government procurement procedures, which compromised timely execution of project activities. However, adoption of a central procurement approach led by TARI–Mikocheni helped other partners to shorten the procedure and fast-track procurement by effecting payment centrally and directing shipment to the respective countries.

The success of the CDP was built through understanding of diversity of capacities and tapping into comparative advantages between partners. There was an obvious difference in capacity between East and Southern African institutions and overseas partners (NCSU and Rutgers University) which was revealed by differences in implementation of the project activities. However, through annual collective planning, sharing of expertise, technical backstopping, exchange visits and specific tailor-made training enabled building capacity, sense of ownership and strong relationships within partners and led to a successful realization of project outputs and outcomes and build stronger networks within the region.

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Acknowledgements

This work was supported by the Bill & Melinda Gates Foundation (BMGF) and the Department for International Development (DFID) of the United Kingdom through the 'Cassava disease diagnostics for sustainable productivity in Africa' project (Grant no. 51466), coordinated by the Tanzania Agricultural Research Institute (TARI)–Mikocheni, Tanzania.

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UGANDA

Titus Alicai[1], Geoffrey Okao-Okuja[1], Phillip Abidrabo[1], Resty Nanvubya[1], Lilliane Kiiza[1], Peter Sseruwagi[2], Fred Tairo[2] and Joseph Ndunguru[2]

[1] National Crop Resources Research Institute, Namulonge, P.O. Box 7084, Kampala, Uganda
[2] Tanzania Agricultural Research Institute (TARI)–Mikocheni, P.O. Box 6226, Dar es Salaam, Tanzania

Abstract

The Cassava Diagnostics Project (CDP), 2008–2018, was implemented in Uganda through the National Crop Resources Research Institute (NaCRRI). A number of objectives were set, and the milestones prescribed were achieved. To meet our aim of assessing the prevalence of Cassava mosaic disease (CMD), Cassava brown streak disease (CBSD) and whiteflies, we surveyed 1320 cassava fields in 20 districts across the country. We collected 12,000 CBSD virus samples, 2880 CMD virus samples and 288 vials of *Bemisia tabaci* isolates – each vial containing approximately 50 isolates. These were used for molecular analysis with the aim of detecting the virus and whitefly species that occur in Uganda.

Analysis of the field data showed an increase in CMD incidence (18.2–32.5%), a slight decline in CBSD incidence (34–24%) and an increase in *B. tabaci* abundance (8.4–21 per plant). The survey results of 2017 showed that CMD incidence was highest in the western region (45.1%) and lowest in the north (26.3%). The CBSD incidence was highest in the central region (27.6%) and lowest in the western region (16.9%). These trends were observed throughout the project period.

Our characterization of viruses and vectors did not reveal any new species in Uganda. We found two cassava mosaic begomovirus (CMB) species: *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV). The predominant species among these was ACMV (80.9% of samples) and EACMV was present in 19.1%. Among the cassava brown streak viruses (CBSVs) detected, *Cassava brown streak virus* (CBSV) was more prevalent (50.8%) than *Ugandan cassava brown streak virus* (UCBSV) (49.2%). Full genome analysis of the CBSVs showed that CBSV was more variable than UCBSV. It had more positively-selected sites and was evolving five times faster than UCBSV. Seven species of *B. tabaci* were detected, among which SSA1-SG1 (66.3%) was dominant and widely distributed in the country. No new insect vector was identified among the suspect insects studied for potential transmission of CBSVs and CMBs.

Outreach activities to support conventional breeding efforts facilitated the release of two new varieties, NAROCASS1 and NAROCASS2, and the promotion of 374 clones to the next breeding levels. Twenty-five demonstration gardens were established, where 518 farmers were trained. In addition, 160 bags (80,000 mini-stem cuttings) of improved cassava planting materials sufficient to plant 20 ha were produced and distributed to farmers. Over 19,000 copies of various CMD and CBSD awareness materials were distributed to farmers and stakeholders at various fora within the country. In total, 112 extension workers and 419 students were trained on various aspects of the cassava value chain.

Seven project staff participated in five international and national fora, during which project results were disseminated.

The project aimed at strengthening Ugandan scientists, extension workers and farmers to conduct cassava disease surveillance and diagnostics. The CDP project enhanced the existing infrastructure of NaCRRI by constructing one screenhouse used for experiments – a facility which is being shared by other projects. Project staff attended five training visits and 12 meetings. The CDP team also hosted scientists from partner countries who came to monitor project progress and for consultation. As part of CDP, we also provided training to postgraduate students during their studies.

Acronyms and abbreviations

5CP	Cassava Varieties and Clean Seed to Combat CBSD and CMD Project
A2N	Africa 2000 Network Uganda Limited
ACMV	*African cassava mosaic virus*
BUCADEF	Buganda Cultural and Development Foundation
CBB	Cassava bacterial blight
CBSD	Cassava brown streak disease
CBSV	*Cassava brown streak virus*
CDP	Cassava Diagnostics Project
CMD	Cassava mosaic disease
CMB	Cassava mosaic begomovirus
EACMV	*East African cassava mosaic virus*
MAP	Months after planting
mtCOI	*Mitochondria cytochrome oxidase* gene
NaCRRI	National Crop Resources Research Institute
TARI	Tanzania Agricultural Research Institute
UCBSV	*Uganda cassava brown streak virus*
UNFFE	Uganda National Farmers Federation
UWA	University of Western Australia
WAVE	West African Virus Epidemiology project

Results summary: Uganda

Aim I: Understand the threat from evolving viruses and vectors	
Objective 1: Disease epidemiology	
Disease and whitefly prevalence surveys conducted	• Three surveys were conducted in 2013, 2015 and 2017 in which 400 cassava fields were surveyed in each year. • Mean Cassava mosaic disease (CMD) incidence (%) and severity were: 18.2 & 2.7, 21.6 & 2.6 and 32.5 & 2.8 in 2013, 2015 and 2017, respectively. • Mean Cassava brown streak disease (CBSD) incidence (%) and severity were 34.0 & 2.2, 21.0 & 2.4 and 24.0 & 2.4 in 2013, 2015 and 2017, respectively. • Mean whitefly populations/field during survey years were 8.4, 34 and 21 in 2013, 2015 and 2017, respectively.
Objective 2: Characterization of emerging viruses	
Cassava virus isolates in the project countries sequenced and analyzed	• Cassava brown streak virus (CBSV) isolates (50) were deep sequenced at Biosciences eastern and central Africa Research Institute, Nairobi, Kenya and the sequences analyzed at the University of Western Australia (UWA). • Three CBSV full genome sequences were published in Alicai et al. (2016).
Cassava virus distribution maps for partner countries, generated (incidence, severity, whitefly, viruses, sat)	• From the surveys conducted in 2009, 2013, 2015 and 2017, 27 different maps were produced. These included CMD and CBSD incidence maps (six), CMD and CBSD severity maps (six), CMD and CBSD prevalence maps (two), CMD infection type maps (three), whitefly abundance maps (four) and CMD and CBSD virus distribution maps (six).
Objective 3: Characterization of disease vectors	
Whiteflies characterized	• Sixty-four consensus sequences of *mitochondria cytochrome oxidase* (*mtCOI*) gene sequences were generated from the Uganda isolates. From these, seven species of whitefly (*Bemisia tabaci*) were identified and two papers published in 2012 and 2018. • Whitefly *mtCOI* data published in two papers by Mugerwa et al. (2012, 2018).
Potential insect vectors of CBSVs identified	• Seventy-three isolates (10 species) of insects screened for CBSVs and Cassava mosaic begomoviruses (CMBs). • None of the insect species confirmed with CBSVs or CMBs.

Aim II: Support clean seed systems for farmers	
Objective 6: Conventional breeding support	
Breeders' material monitored for disease and indexed for virus (CMBs and CBSVs) load at 3, 6, 9 and 12 MAP	• Out of the 675 genotypes tested, 607 had no CMD and were promoted for CMD management. • There were 248 genotypes with no CBSD and these were promoted for CBSD management. Virus loads were not determined.
Farmers trained on CMD and CBSD disease symptom recognition and management strategies	• There were 1034 farmers (547 females and 487 males) trained in the demonstration sites, agricultural shows and during 2013, 2015 and 2017 disease surveillance surveys and/or in workshops.
Demonstration plots for benefits of using virus-indexed planting materials established on-farm	• During 2014–2017, 25 demonstrations established on-farm, and persons trained totaled: farmers (905), extension workers (112) and students (419) trained at various fora across the country.
Information materials developed and disseminated	A total of 19,200 copies of dissemination materials printed in English, Luo, Luganda and Ateso languages. Of these: • 500 T-shirts on CBSD awareness printed and distributed to stakeholders • 16,700 brochures on CMD and CBSD disseminated to cassava farmers • 1500 posters on CMD and CBSD disseminated to cassava farmers • 500 copies of cassava seed manuals disseminated to stakeholders • Three project presentations made at sister research institutes and other stakeholders • During the project period, three manuscripts were published, one submitted for publication and three in preparation.
Aim III: Build sustainable regional capacity	
Objective 10: Strengthening stakeholder linkages	
Awareness on availability of diagnostic capacities created through training and different media	• Two awareness programs on the availability of diagnostics capacity and processes were produced and aired on URBAN and NBS TV in 2014 and 2016, respectively.

Objective 11: Strengthening human capacity and infrastructure	
Human capacity	
Project staff recruited	• A total of six project staff were recruited and remunerated: one Team Leader with roles of general project management; one Assistant Team Leader to assist with project management; two Research Assistants to plan, execute and supervise project activities in the field; and two Research Technicians to conduct laboratory diagnostics.
PhD and MSc trained on different aspects of cassava virus diseases	• One PhD and one MSc student trained at Greenwich University, UK, and Jomo Kenyatta University of Agriculture and Technology, respectively. Both completed in 2017.
Advanced specialized training and visits for project scientists (1-2 months) conducted	• Dr Titus Alicai visited Dr Laura Boykin at UWA for technical training on bioinformatics in December 2015 for one month.
Extension workers, crop inspectors and other stakeholders (1 week) training	• Four workshops conducted during 2014–2016. In 2014, 2015 and 2016, there were 24, 47 and 41 extension workers trained, respectively.
Infrastructure strengthening	
Greenhouses constructed/renovated	• One screenhouse completed and being used for conducting experiments that require keeping away unwanted insects or containment of wanted/introduced insects.

Background

Cassava (*Manihot esculenta* Crantz) is the second most important crop after bananas in Uganda. Some 500,000 hectares are under cassava cultivation and the most recent published information puts cassava production at 5.4 Mt, representing an average yield of 12 t/ha (FAO, 2014). The crop is grown by over 75% of households and contributes about 22% of cash income to rural farmers. The leading cassava producing areas are eastern, northern, central and western regions of the country. However, cassava production is constrained by several insect pests and diseases, the most important of which are cassava mosaic disease (CMD) and cassava brown streak disease (CBSD).

Following the severe outbreak of CMD in Uganda in the 1990s and CBSD in 2004, there have been efforts to counter their spread within the country. Cassava mosaic begomoviruses (CMBs) cause CMD, while CBSD is caused by cassava brown streak viruses (CBSVs). Both these major diseases are transmitted by the whitefly vector, *Bemisia tabaci*, and spread by widespread use of infected planting materials (Legg et al., 2015; Rey & Vanderschuren, 2017).

The epidemic of CMD associated with high abundance of *B. tabaci* reported in the 1980s in Uganda (Otim-Nape et al., 1994, 1998) has spread extensively within the country and beyond its borders, affecting other countries in the region – for example, Tanzania, Rwanda, DR Congo and South Sudan Kenya. The CMD epidemic was due to a virulent recombinant virus strain, the *East African cassava mosaic virus*-Uganda variant (EACMV-UG). In 2004, symptoms resembling CBSD were reported on cassava in central Uganda and tests on the diseased materials confirmed the presence of CBSD caused by CBSV (Alicai et al., 2007). Since then, CBSD has continued to spread widely in the country and our studies have shown that its prevalence in the country is at >90%, although incidence remains low.

As part of efforts to mitigate their effects and to guide control intervention, surveys have been conducted annually to monitor changes in the incidence, severity and spread of these major diseases and pests. Additional efforts during the course of the Cassava Diagnostics Project (CDP) project have included knowledge building by reaching farmers directly and through partners; working on the development and deployment of improved cassava varieties; and developing infrastructure and human resource capacity. To achieve the CDP project goals set for participating countries, studies were conducted within Uganda for eight years and were guided by the targets and milestones set by the CDP project. In this document, we report the findings, achievements and challenges of the project in Uganda during 2009–2017.

SECTION ONE: Understanding the threat from evolving viruses and vectors

Disease epidemiology in Uganda

Four pest and disease monitoring surveys were conducted in the cassava-growing regions in Uganda in 2009, 2013, 2015 and 2017. A total of 20 districts were surveyed: Apac, Lira and Pader (northern); Bugiri, Kamuli, Kaberamaido, Kumi, Mayuge, Pallisa and Soroti (eastern); Kayunga, Kiboga, Luwero, Mubende, Mukono, Nakasongola and Wakiso (central); and Hoima, Masindi and Kibaale (western) regions. During these surveys, a total of 1320 farmer fields were assessed.

The aim of the surveys was (1) to determine and map the incidence and severity of CMD, CBSD and whiteflies and (2) to determine the geographical distribution of CMBs and CBSVs and *B. tabaci* species in the surveyed areas. The survey results showed that CMD and CBSD were present in all districts surveyed, that they are still a threat in the country and that a concerted effort is needed for the effective management of these diseases and their vectors.

CMD and CBSD incidence

Overall CMD incidence was 25.7%, 18.2%, 10.6% and 25% in 2009, 2013, 2015 and 2017, respectively, indicating an increase in incidence (Figure 1). The CBSD incidence was 11.2% in 2009 and rose to 34% in 2013, reduced to 21% in 2015 and rose slightly to 24% in 2017. A rise in CMD and a reduction in CBSD incidence could be due to farmers paying more attention to CBSD, a new disease with more devastating effect than CMD.

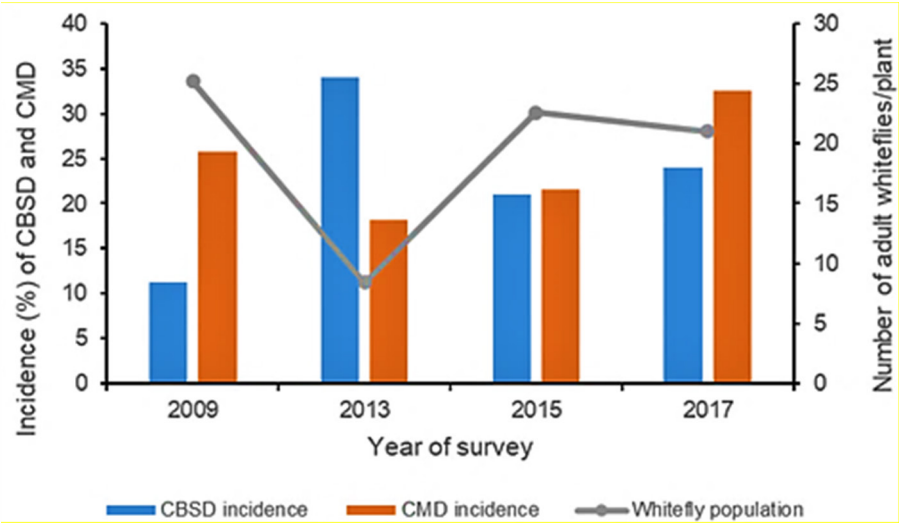


Figure 1 CMD and CBSD incidence and whitefly abundance trends, 2009–2017

CMD and CBSD severity

Symptom severity of CMD showed a slight increase from 2.2 (using a 1–5 scale, with 0 = no symptoms and 5 = very severe symptoms) in 2009 to 2.6 in 2017; however, CBSD severity was 2.4 in 2009, reduced to 2.2 in 2013, and slightly increased in 2017 to 2.4, indicating mild disease in the

surveyed years. In the four years of the survey, cutting-borne infection (52.6%) was higher than whitefly-borne infection (47.4%), showing that the disease was perpetuated mainly by infected planting materials. This was probably due to farmers' limited knowledge on how the disease spreads or due to lack of clean planting materials within communities that resulted in recycling of infected planting materials.

Whitefly abundance

Mean whitefly abundance was highest in 2009 at 25.2 adults per plant. This was lower in 2013 with 8.4 and increased in 2017 to 21.0 adults per plant (Figure 1).

Prevalence of improved cassava varieties in the surveyed areas

A number of improved cassava varieties and local varieties ('landraces') were cultivated across the surveyed areas. Overall, the prevalence of improved varieties was 68.7% in 2009, 70.0% in 2013, 62.5% in 2015 and 65.7% in 2017 giving a mean prevalence of 66.7% in the four years (Table 1). Despite the high prevalence of improved varieties, landraces were also preferred widely by farmers probably due to their good attributes and farmer preferred qualities.

Table 1 Prevalence of improved varieties 2009–2017

Year of survey	Improved variety prevalence (%)
2009	68.7
2013	70.0
2015	62.5
2017	65.7
Mean	**66.7**

Alternative hosts for CBSVs and CMBs and associated insect vectors identified

Work on alternative hosts of CBSVs and CMBs was not the mandate of CDP in Uganda; however, a survey was carried out in 2016 to collect wild plant species considered as potential virus reservoir hosts. This was a joint survey involving the CDP-Tanzania and CDP-Uganda teams, and participants from the National Crops Resources Research Institute (NaCRRI). The goal was to unravel virus diversity in reservoir plant species that may pose risks to cassava production and agriculture in general. During the survey, non-crop plants at 53 different sites were assessed for virus symptoms. A total of 235 symptomatic leaf samples were collected and taken by the CDP-Tanzania team for processing using molecular techniques.

Characterization of emerging viruses

Cassava virus isolates sequenced and analyzed

It is not known if CMBs and CBSVs have genetically changed, with possible emergence of more virulent strains. There was therefore a need for regular collection of CMD and CBSD virus samples from farmers' fields and to test them to identify the virus species present, with subsequent sequencing of the isolates in an advanced laboratory to characterize them.

During the monitoring surveys, virus samples were collected from each of the 1320 fields assessed in the country. A total of 1840 CMD and 9200 CBSD samples were collected in the four years of surveys. Representative samples were picked from each year's collection, from which DNA was extracted using a method described by Dellaporta et al. (1983), and RNA was extracted using the CTAB method (Lodhi et al. 1994). The samples were subsequently tested for the presence of CBSVs using primers, which simultaneously amplify *Uganda cassava brown streak virus* (UCBSV) and CBSV at 437 and 343 bp, respectively (Mbanzibwa et al., 2011), and CMBs by primers that amplify ACMV at 1000 bp and EACMV-UG at 1500 bp (Harrison et al., 1997). The samples that reacted positively to each of the above tests were selected and sequenced.

Detection of CMD and CBSD viruses

From the diagnostics results, there was random distribution of CMBs in the surveyed areas. The results further showed that in 2013, ACMV (70%) was the most common virus species; however, in 2015, mixed infections of ACMV and EACMV-UG2 (56%) were the most prevalent. The diagnostic results further revealed that UCBSV was the most common virus species in the country with an increase in prevalence from 48% in 2013 to 63.7% in 2015. However, this further changed in 2017 with CBSV being more predominant even on a regional basis: ACMV predominated except in the northern region where there was a shift from ACMV being predominant in 2013 to dual infection predominating in 2017. For CBSVs, there was a shift from UCBSV being the predominant virus in 2013 to CBSV predominating in 2017 (Figure 2). These results indicate a marked change from the situation in Uganda at the height of the CMD epidemic in the 1990s, when EACMV-UG2 was the most prevalent virus species/strain and had devastating effects on cassava production.

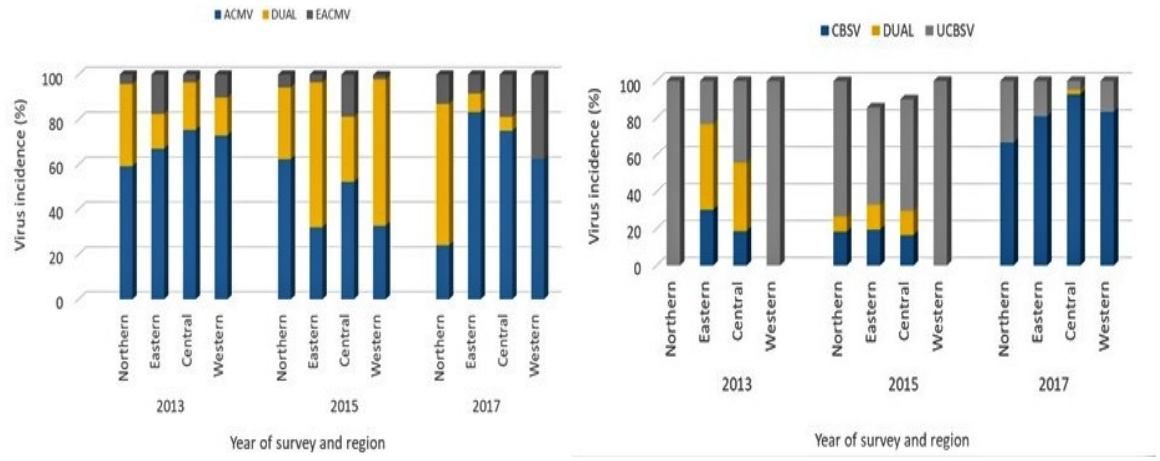


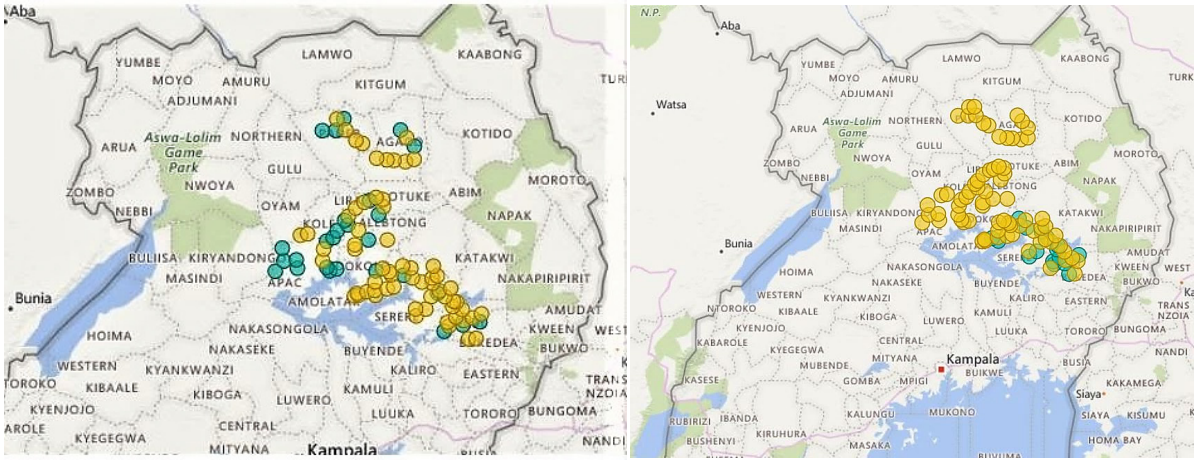
Figure 2 CMBs and CBSVs occurring in Uganda during 2013–2017

Sequence analysis of CMBs and CBSVs

From the 2013 and 2015 surveys, 133 CMB and 128 CBSV isolates were selected and sequenced at Macrogen (The Netherlands) using Sanger techniques. From these isolates, 146 CMB and 156 CBSV sequences were generated, *de novo* assembled and edited. From the edited sequences, 63 CMB and 57 CBSV consensus sequences were generated and phylogenetic trees constructed. A further 40 CMB isolates collected in 2015 were submitted for direct whole genome sequencing in South Africa in January 2016. Our CMB sequencing results revealed the presence of ACMV and EACMV-UG2. Analysis of the CBSV and UCBSV genomes showed that CBSV was more variable, had more positively-selected sites and was evolving five times faster than UCBSV (Alicai et al., 2016).

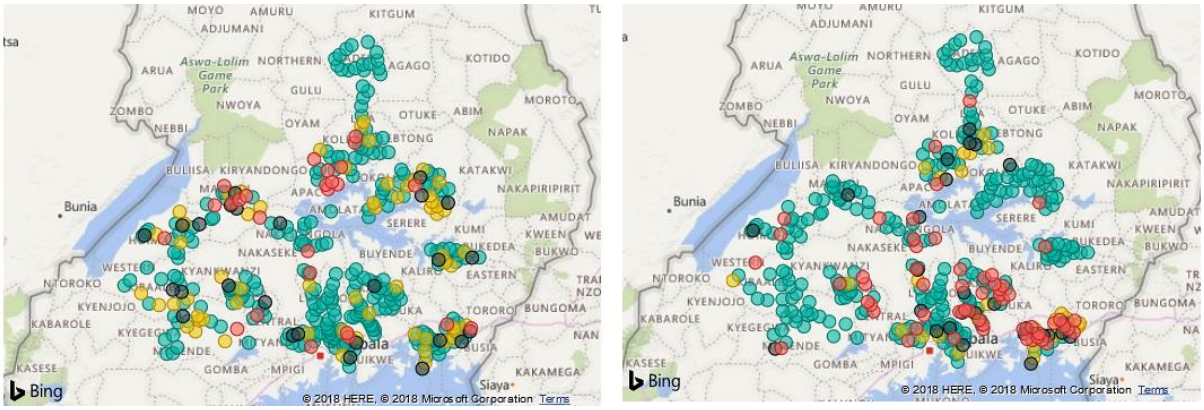
Cassava virus distribution maps for partner countries generated (incidence, severity, whitefly, viruses)

During the surveys conducted between 2009 and 2017, 26 maps from the survey results were produced and shared with Tanzania Agricultural Research Institute (TARI)–Mikocheni and partners. The various maps developed included the following: CBSD, CMD and Cassava bacterial blight (CBB) incidence maps; CBSD, CMD and CBB severity maps; CBSD and CMD prevalence maps; CMD infection types; whitefly abundance maps; CBSD and CMD virus distribution maps; and whitefly species distribution maps as shown in Figure 3 and Figure 4 below. These maps informed researchers on CBSD, CMD and whitefly hotspots. This information provided guidance on the strategic deployment of materials, especially where to test materials being developed by breeders, and where to set up seed multiplication blocks and demonstration gardens as farmer training sites.



= 0–25% (Low) >75–100% (Very high)

Figure 3 Disease incidence in locations surveyed in 2009 for CMD (left) and CBSD (right)



= 0–25% (Low) >25–50% (Medium) >50–75% (High) >75–100% (Very high)

Figure 4 Disease incidence in locations surveyed in 2015 for CMD (left) and CBSD (right)

Characterization of disease vectors

Whitefly

A total of 320 vials, each containing about 50 adult (dead) whitefly samples, were collected on cassava during the surveys for characterization and determination of the genetic diversity of the mitochondrial cytochrome (*mtCOI*) gene through sequencing. The DNA was extracted from single

insects. The PCR amplification of the *mtCOI* gene fragment was achieved with the primer pair MT10/C1-J-2195 and MT12/L2-N-3014 (Simon et al., 1994) for a fragment of 850 bp. Altogether, 140 whitefly samples were sequenced by Macrogen Inc, The Netherlands, using the Sanger technique.

Sequence analysis

There were 230 sequences generated from the 140 isolates sequenced; 80 sequences were *de novo* assembled and edited. From the 80, 63 consensus sequences were generated, and one phylogenetic tree constructed. The results showed the occurrence of six *B. tabaci* species in Uganda: SSA1-SG1, SSA1-SG2, SSA2, Mediterranean, Indian Ocean and Uganda species (Table 2). The SSA1-SG1 (66.3%) was predominant on cassava; SSA2 comprised 29.4% of the species and was predominant in central and north eastern Uganda. The Mediterranean species was predominant on the weeds evaluated. One instance each of the Indian Ocean and the Ugandan species were also found on non-cassava plant species.

Table 2 Distribution of whitefly (*Bemisia tabaci*) species from the 2015 survey. '–' indicates not found

Region	Whitefly species					
	SSA1-SG1	**SSA1-SG2**	**SSA2**	**Indian ocean**	**Mediterranean**	**Uganda**
Northern	20.4	–	3.3	–	–	–
Eastern	20.4	–	19.6	–	–	1.0
Central	25.5	1.3	6.5	1.0	1.0	–
Western	–	–	–	–	–	–
Total	**66.3**		**29.4**	**1.0**	**1.0**	**1.0**

Potential insect vectors of CBSVs identified on cassava

The identity and diversity of other potential cassava virus vectors were studied using insect specimens collected in the major cassava-growing districts in each of the four regions of the country in 2014 and 2015. The surveys targeted and collected insects found feeding on cassava plants; which were picked and preserved in 80% ethanol.

There were 73 insect isolates collected and identified to species level by Dr Andrew Polaszek, an agricultural entomologist at the Natural History Museum in London, UK. The results from the 2014 survey showed that none of the insects collected could be virus vectors because most were generalist predators that include *B. tabaci* in their diet. The very small coccinellid beetles identified were specific whitefly predators that fed on *B. tabaci* (Table 3). In 2015, 360 vials containing different samples of insects were collected. The insects collected were grouped based on their morphological similarities.

Table 3 Insects identified and tested for potential to carry virus, 2014

Group number	Insects identified by group
1	*Camponotus* sp. (ants)
2	Coccinellidae (beetles)
3	Coccinellidae larvae
4	Tettigoniidae (bush crickets)

Group number	Insects identified by group
5	Spiders
6	Lymantriidae (caterpillars)
7	Forficulidae (earwigs)
8	*Trigona* sp.
9	Lygaeidae
10	Pentatomidae

SECTION TWO: Integrated pest management

Supporting clean seed systems for farmers

Conventional breeding support

Breeders' material monitored for disease and indexed for virus (CMBs and CBSVs)

Since the outbreak of the CMD epidemic in the country in the 1990s, and more recently the CBSD epidemic, several breeding efforts were initiated to develop resistant cassava varieties to manage the viral diseases affecting the crop. The CDP supported efforts for screening breeders' materials for cassava viruses and the virus titers. The data obtained were used together with the disease symptom assessments to guide and/or confirm resistance to viral disease.

In 2014, three breeding trials were monitored: (1) Uniform Yield Trials (UYTs) using four genotypes, (2) CBSD screening trials using 11 clones and (3) CBSD-resistance crossing block using 127 clones. In total, 324 CMD- and 1641 CBSD-symptomatic leaf samples were collected and analyzed using standard PCR protocols. The results were as follows: in the UYTs, all samples (100%) tested negative for CMD, and 57.8% of samples tested positive for CBSD viruses. In the CBSD screening trials, of the 11 clones, five (45.4%) tested negative for CBSD but all 11 (100%) tested negative for CMD viruses. In the CBSD crossing block of 217 clones, 83 (38.2%) had no visual CBSD symptoms, and 92 (42.4%) tested negative for CBSVs. From the same trial, 80 (36.9%) clones tested positive and 137 (63.1%) tested negative for CMD viruses.

From the CBSD screening trials, five clones (out of 11) were selected for multi-locational trials; from the CBSD-resistance crossing block, 85 clones were promoted to advanced seedling trial; and from the UYT trials, disease data supported the release of two genotypes, NAM130 and MM06/0130, as new varieties, named NAROCASS1 and NAROCASS2, respectively. The varieties with very low disease incidence or that tested negative to the viruses were selected for release or promotion to the next level of research.

In 2015, two genotype-by-environment (G×E) trials were monitored at two locations, where genotype performance was evaluated within a specific environment. The G×E trials under the NextGen project comprised 650 clones, while those under the Cassava Varieties and Clean Seed to Combat CBSD and CMD Project (5CP) project had 25 clones – thus, a total of 675 clones were monitored. From these trials, 94 CMD and 1308 CBSD virus isolates were collected and tested. Data from these trials showed that 607 (93%) clones exhibited no CMD symptoms and 248 (38%) exhibited no CBSD symptoms. The 248 clones without both CMD and CBSD symptoms were advanced to participatory variety selection trials for further evaluation. These are still being monitored, with promising results suggesting that they are candidates for resistance to CMD and CBSD and probable release.

Table 4 Breeders' materials monitored for disease and viruses in 2014 and 2015

Description	Number of trials monitored per year	
	2014	2015
No. of locations	4	4
No. of trials monitored	3	2
Type of trial	UYT (4 genotypes) CBSD screening trial (11 clones) CBSD resistance crossing block (127 clones)	G×E (NextGen) 654 clones G×E (5CP) 25 clones
No. of samples tested	CMBs: 324 CBSVs: 1641	CMBs: 94 CBSVs: 1308

Reaching farmers directly and through partners

Training farmers on CMD and CBSD disease symptom recognition and management strategies

It was a requirement of the project that farmers must either be reached directly or through partners such as extension workers. This was intended to increase farmers' knowledge on cassava pests and disease management as well as their access to quality planting materials to improve cassava production and productivity at the farm level.

Between 2014 and 2017, CDP-Uganda trained a total of 1034 farmers (487 male and 547 female) at five demonstration sites. The training covered good agronomic practices, pest and disease identification and management strategies, stem storage, weed management regimes, post-harvest handling and cassava value addition to prolong shelf-life of cassava and cassava products for better markets. The trained farmers are now using the acquired knowledge within their farming communities.

Table 5 Farmers trained from 2014 to 2017 – by gender

Year	Male	Female	Total
2014	28	33	61
2015	180	230	410
2016	30	15	45
2017	249	269	518
Total	**487 (47.1%)**	**547 (52.9%)**	**1034 (100%)**

Cultivating demonstration plots to underline the benefits of using virus-indexed plant material

Following the project protocol, demonstration gardens were created. In each location, improved cassava varieties were planted together with a local variety preferred by the farmers. This activity was intended to show farmers the benefit of using disease-free planting materials when establishing

their fields. These demonstration gardens also acted as farmer training centers. The CDP partners and sponsors in this endeavor included the Uganda National Farmers Federation (UNFFE), the Africa 2000 Network Uganda Ltd (A2N), OXFAM and the Buganda Cultural And Development Foundation (BUCADEF).

Between 2014 and 2017, in partnership with other stakeholders, a total of 25 demonstration plots were established in 11 districts across the country, 20 of which were harvested (Table 6). The demonstration plots were established with three improved virus-indexed cassava varieties (NAROCASS1, NASE14 and NASE19) and one farmer-preferred local variety as identified by the farmers in each location.

During the period of growth, the demonstration plots were monitored for CMD, CBSD and whiteflies at intervals of 2, 5, 8 and 12 months after planting (MAP); both researchers and farmers participated in monitoring the demonstration plots. All of the established demonstration gardens were evaluated and harvested. At harvest, the farmers were invited to participate in a 'sensory testing' exercise. This meant tasting raw and cooked cassava roots first to identify the sweet and bitter varieties and second to indicate other attributes that they may or may not have liked in a variety. This exercise was used to guide the breeders on how to improve the rejected variety.

Some 205,200 mini-stem cuttings were produced during 2014–2017 from the demonstration sites for distribution to farmers and their communities. These cuttings enabled the farming communities to plant approximately 20 ha of cassava (Table 6).

Table 6 Demonstration-plot experiments 2014–2017

Start of experiment	Number of plots created	Number of plots maintained to end of experiment	Sponsor	Partner
2014	2	2 (2015)	CDP	–
2015	4	4 (2016)	UNFFE	CDP
2015	8	8 (2016)	A2N/OXFAM	CDP
2016	6	6 (2017)	A2N/OXFAM	CDP
2016	5	Experiment not completed	CDP	BUCADEF
Total	**25**	**20**		

Figure 5 Participatory monitoring of demonstration gardens: researchers Geoffrey Okao-Okuja (fourth from left) and Lilliane Kiiza (second from right)

In the demonstration plot evaluations, NAROCASS1 performed best in terms of high yield and disease resistance, followed by NASE14. However, in Hoima, the landrace yield exceeded that of NASE14 and NASE19 (Figure 6), possibly due to the low disease pressure in the area. Generally, farmers had a very high perception of the improved varieties due to their disease resistance and high yield. They also believed that improved varieties together with the knowledge acquired would increase cassava production when they saw the yield gain resulting from improved varieties. Demonstration plots also positively changed farmers' attitudes toward growing cassava – a crop which was being abandoned by some communities due to poor yields. Yield examples from Oyam and Pader demonstration sites are shown in Figure 7.

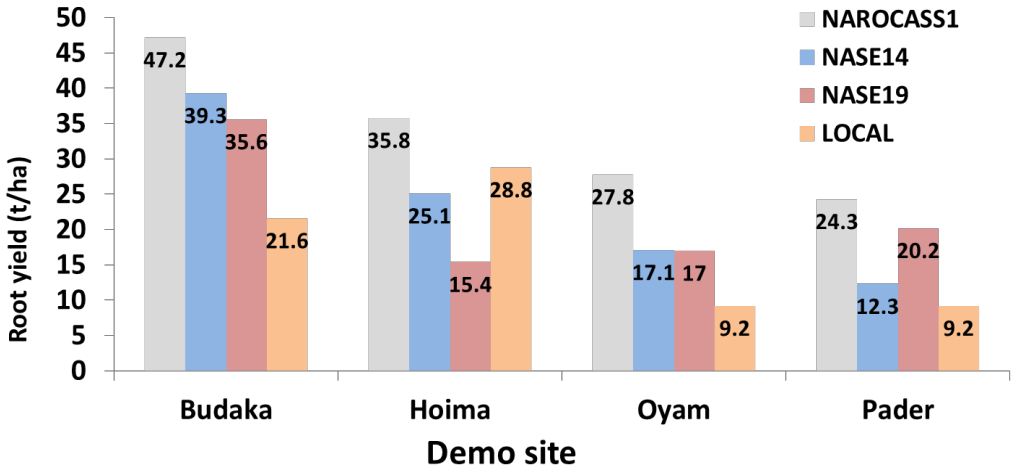


Figure 6 Yield of cassava varieties harvested from demonstration plots in 2016

Figure 7 NAROCASS1 and NASE19 yield at Oyam and Pader in 2016

Table 7 Incidence and symptom severity of CMD and CBSD in the demonstration plots, 2016. Inc = incidence; Sev = severity on a scale of 1–5 (1 = no symptoms and 5 = very severe symptoms)

Variety	Budaka		Hoima		Oyam		Pader	
	Inc (%)	Sev	Inc (%)	Sev	Inc (%)	Sev	Inc (%)	Sev
CMD								
NAROCASS1	0	1	0	1	13.0	2.7	0	1
NASE14	0	1	0	1	0.5	2.0	0	1
NASE19	0	1	0	1	0	1	0	1
Local	86.2	2.3	0	1	73.0	3.2	52.5	3.0
CBSD								
NAROCASS1	0	1	0	1	0	1	0	1
NASE14	1.5	2.0	0	1	0	1	0	1
NASE19	0	1	0	1	0	1	0	1
Local	2.3	2.0	0	1	0	1	0	1

Development and dissemination of information materials

Training

Eleven training sessions were carried out at demonstration sites at 2, 5, 8 and 12 MAP. Training was conducted on site selection, land preparation, selection and proper storage of planting materials, and cassava pest and disease symptom recognition and management. At harvest time, farmers were trained on post-harvest handling and management – as shown in Figure 8.

Figure 8 Mr Geoffrey Okao-Okuja training farmers in Lira 2014 (left) and in Oyam in 2015 (right)

Some 19,000 copies of various information materials – on cassava agronomy and cassava pests and disease symptom recognition and management – were developed and distributed to farmers. During the second training session at 2 MAP, a questionnaire was administered by the CDP-Uganda team and completed by farmers to assess the farmers' initial level of knowledge on cassava pests, diseases and management. A total of 681 farmers were trained, 50.8% (346) of whom were female (Table 8).

Table 8 Farmers trained in 2014–2017 by gender

Year	Male	Female	Total
2014	28	33	61
2015	180	230	410
2016	30	15	45
2017	97	68	165
Total	**335 (49.2%)**	**346 (50.8%)**	**681 (100%)**

Disease awareness

Information materials on CMD and CBSD were integral in imparting knowledge to farmers and their communities. To achieve this, 19,200 copies of several information materials were developed and disseminated to farmers and other stakeholders during 2014–2017 (Table 9). The materials included brochures, posters, seed manuals and T-shirts for the CMD and CBSD awareness campaign. The materials were printed in English, Luo, Luganda and Ateso. The themes of the awareness campaign were "Use of clean planting materials against viral diseases of cassava" and "Facts about CBSD and CMD". The materials were distributed at different fora within the country.

Table 9 Information materials developed and disseminated, 2014–2017

Year	Material type	Number of items
2014	Cassava seed quality manual	500
2014	T-shirts on CBSD awareness	500
2014	CBSD awareness brochure	15,000
2015	CBSD awareness poster	500
2016	CBSD awareness poster	500
2017	CBSD awareness poster	500
2017	CMD awareness brochure	200
2017	CBSD awareness brochure	1500
Total		**19,200**

Building sustainable regional capacity

Strengthening stakeholder linkages

Stakeholder engagement

From the start of CDP implementation in Uganda, a number of stakeholders were visited and contact maintained throughout the project. These interested parties ranged from university departments to research institutions and government departments (see Table 10).

Table 10 Partners and stakeholders visited during the impact assessment baseline study and monitoring and evaluation missions for the 'Disease diagnostics for sustainable cassava productivity in Africa' project, 2014

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
National Crop Resources Research Institute (NaCRRI)	Namulonge	NARS (Project partner)	Dr Titus Alicai (Country Team Leader), Mr Geoffrey Okao Okuja (Assistant Team Leader), Ms Lillian Nanvubya (Technician), Mr Godfrey Sseremba (Research Assistant)	Hosting the project, breeding for resistance to CMD and CBSD and disease diagnostics	Dr Titus Alicai Mr Geoffrey Okao Okuja Mr Godfrey Sseremba
		Cassava Breeder	Dr Antony Pariyo	Breeding cassava for resistance to CMD and CBSD	Dr Antony Pariyo
		BMGF supported project on cassava seed systems	Dr Antony Pariyo (Project Coordinator)	Production and distribution of disease-free planting materials	Dr Anthony Pariyo
Ministry of Agriculture, Animal Industry and Fisheries (MAAIF)	Entebbe	Government	Ms Florence Tumuboine (Assistant Commissioner)	Plant health, seed certification and regulation	Ms Florence Tumuboine
Nakasongola District Farmers Association (NADIFA)	Nakasongola	Non-governmental Organization (NGO)	Mr Joshua Ssebwato (Extension worker)	Seed multiplication and distribution	Mr Joshua Ssebwato

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
Bio-Crops (U) Limited, Kampala	Kawanda	Private tissue culture laboratory	Dr David Talengera (Technical Director)	Production of disease-free planting materials using tissue culture	Dr David Talengera
Makerere University, Department of Agricultural and Environmental Sciences	Kampala	University (Collaborators)	Dr Ssetumba Mukasa, (Lecturer/ Acting Head of Department of Agricultural and Environmental Sciences), Dr Mildred Ochwo-Ssemakula (Lecturer), Dr Jeninah Karungi (Lecturer)	Training and student supervision	Dr Mildred Ssemakula
Farmer	Nakasongola	Farmer	Ms Justina Ssenku, Leader of farmer association NADIFA	Cassava production and seed multiplication	Ms Justina Ssenku

In order to strengthen stakeholders' linkages, awareness of NaCRRI's diagnostic capacity was created through television talk shows during 2014–2017. The television programs were aired on local TV stations after journalists visited NaCRRI and interviewed project scientists. The topics discussed included strategies such as selection of clean planting materials to establish new crop fields, early removal of diseased plants within established crop stands and the management of the major cassava diseases and pests prevalent in the country. They also included the diagnostic processes carried out in the molecular biology laboratory at NaCRRI. An interview on diagnostic processes was broadcast by URBAN TV on 2 July 2014, and an interview on the availability of diagnostics capacities was broadcast by NBS TV in January 2016.

During 2016–2017, NaCRRI continued to collaborate with UNFFE, A2N, Oxfam and BUCADEF to scale up farmer training, demonstration plots and the multiplication of improved and virus-tolerant or virus-resistant planting materials for viral disease management within the various communities.

Exchange visits between scientists in the CDP partner countries

In 2014, a team of scientists from TARI–Mikocheni visited Uganda to monitor project progress. In October 2016, the project team leader (Dr Titus Alicai) and his assistant (Mr Geoffrey Okao-Okuja) attended the CDP Annual General Meeting in Zambia. In December 2016, two scientists (Mr Phillip

Abidrabo and Mr Geoffrey Okao-Okuja) participated in the Data Entry and Assembly Workshop in Dar es Salaam, Tanzania.

Strengthening human capacity and infrastructure

Project staff recruited in 2013 and 2015

During the project duration, several staff were recruited to provide the project with a range of skills (Table 11).

Table 11 Staff recruitment 2013–2015

Name	Role	Year of recruitment
Titus Alicai	Team Leader	2013
Geoffrey Okao-Okuja	Assistant Team Leader	2013
Godfrey Sseremba	Research Assistant	2013
Lilliane Kiiza	Research Technician	2013
Resty Nanvubya	Research Technician	2013
Brian Kula	Accounts Assistant	2013
Phillip Abidrabo	Research Assistant	2015

Mr Godfrey Sseremba left the project to pursue PhD studies in Ghana and has now returned to work at the National Coffee Research Institute as a Research Officer.

Human capacity

Training for project staff 2013–2017

During 2013–2017, several project staff were trained on various aspects of the cassava value chain as part of the project objectives and goals. This training program included financial management, disease surveys and diagnostic techniques, bioinformatics and general project administration (Table 12).

Table 12 Project staff trained 2013 –2017

Date	Course title	Venue	Staff trained
May 2013	Financial management	Dar es Salaam, Tanzania	Brian Kula
Aug 2014	Disease surveys and diagnostics	Dar es Salaam	Lilliane Kiiza and Resty Nanvubya
Oct 2014	Bioinformatics basics	Kawanda, Uganda	Lilliane Kiiza, Resty Nanvubya and Godfrey Sseremba
Feb 2015	Biosafety	Serere, Uganda	Geoffrey Okao-Okuja
July 2015	Bioinformatics	Australia	Titus Alicai
Oct 2015	Intellectual property rights	Nairobi, Kenya	Geoffrey Okao-Okuja and Titus Alicai
Jan 2016	AgShare, scientific writing & communication	USA	Titus Alicai
Mar 2016	Mobile apps for cassava disease surveillance	NaCRRI, Uganda	Geoffrey Okao-Okuja and Phillip Abidrabo
Nov 2016	Data entry & assembly	Dar es Salaam	Geoffrey Okao-Okuja and Phillip Abidrabo
May 2017	Gender Action Learning Systems (GALS)	Lira, Uganda	Geoffrey Okao-Okuja

Training of students

In addition to project staff and government workers, the project facilities and staff at the molecular biology laboratory at NaCRRI were made available for training other stakeholders, especially students from colleges and universities in the country. During 2013–2017, students were trained for a range of academic qualifications (Diploma, Bachelors, Masters and PhD) on aspects of the cassava value chain with emphasis on molecular diagnostic processes.

A total of 419 students visited the laboratory for practical sessions of one day or longer (Table 13). In addition, in 2015, three project students were attached to the laboratory as part of their course requirement. During the student training, pest and disease symptom recognition, sample collection and storage and the laboratory detection of viruses in cassava samples were the major practical activities.

Table 13 Students trained, 2013–2017

Student category	Number of students
Diploma	16
Bachelors	346
Masters	52
PhD	5
Total	**419**

Extension staff, crop inspectors and other stakeholders (1 week) training

Agricultural extension workers, crop inspectors, district and Ministry of Agriculture staff, and Animal Industry and Fisheries staff were trained on various techniques relating to the cassava value chain. During 2013–2017, a total of 112 of these staff were trained on general cassava production including best agronomic practices, pest and disease management, cassava value-addition and laboratory diagnostic techniques. These training sessions were delivered at NaCRRI, and at Ngetta in Lira district in northern Uganda (Table 14 and Figure 9). These training courses were conducted in partnership with UNFFE and A2N.

Table 14 Extension workers and crop inspectors trained 2014–2017 by gender

Year	Venue	Country of origin	Gender		Total
			Male	Female	
2014	NaCRRI	Uganda	19	5	24
2015	NaCRRI	Uganda	38	9	47
2016	NaCRRI	Somalia	21	4	25
2016	Lira	Uganda	14	2	16
Total			**92 (82%)**	**20 (18%)**	**112 (100%)**

Figure 9 Titus Alicai (left: standing) and Geoffrey Okao-Okuja (right: third from right) at training sessions for extension workers at NaCRRI in 2015

Project staff trained on intellectual property rights, biosafety issues and communication strategies

During 2015–2017, two project staff members received training on intellectual property rights, biosafety issues and communication strategies in Uganda, the USA and in Nairobi, Kenya (Table 15).

Table 15 Staff trained on intellectual property, biosafety and communication strategies in 2015 and 2016

Date	Course title	Venue	Staff trained
Feb 2015	Biosafety	Serere, Uganda	G Okao-Okuja
Oct 2015	Intellectual property rights	Nairobi, Kenya	G Okao-Okuja and T Alicai
Jan 2016	AgShare – scientific writing and communication	USA	T Alicai

Advanced specialized training and visits for project scientists (1–2 months) conducted

Advanced specialized training was attended only by the Team Leader, Dr Titus Alicai, who received training on bioinformatics at the University of Western Australia for one month and also visited the Volcani Center, an agricultural research institute in Israel, for one week to become acquainted with laboratory diagnostic processes.

Project results and information disseminated

During 2013–2017, project staff presented project results and information nationally and internationally in various fora as indicated below.

In July and October 2015 in Jinja and Fort Portal, Kabarole district, two project staff participated in agricultural shows where they informed attendees on the availability of improved and resistant cassava varieties NAROCASS1 and NASE14. They also distributed various posters and brochures on disease awareness.

In June 2016, Titus Alicai and Phillip Abidrabo participated in a virus symposium conference in France where they presented a poster on the "Geographical and genetic diversity of cassava brown streak viruses" in Uganda.

In November 2016, three project staff participated in the NARO–Makerere University joint agricultural dissemination conference. These staff presented a paper and a poster on "CBSD virus diversity" and "Demonstration of the benefits of using virus-indexed cassava planting materials", respectively.

Publications output from the project included work in the following disciplines: virology, entomology and molecular biology. This work was published in international journals such as Nature Scientific Reports (Alicai et al, 2016) and Systematic Entomology (Boykin et a, 2018), attesting to the quality of the science performed by the CDP-Uganda team.

From 2015 to date, project staff have participated in a monthly Root Crops Program seminar at NaCRRI and have occasionally presented information on the project results. Among the topics presented were the "Status of cassava mosaic disease, cassava brown streak disease and whitefly abundance in Uganda" and the "Genetic diversity of cassava brown streak viruses in Uganda" by Mr Geoffrey Okao-Okuja and Phillip Abidrabo, respectively.

Institute Director trained in leadership and management

In July 2014, the NaCRRI Director (Dr James Ogwang) participated in the Leadership and Management Skills training in Kigali, Rwanda.

Infrastructure strengthening

To strengthen infrastructure development, in 2014, one screenhouse was constructed at NaCRRI and is now being used. One plant growth chamber and one portable car freezer were procured. In addition, three laptop computers, one desktop computer and one printer were procured for the project and are being used by the NaCRRI community for various purposes (Table 16). Laboratory reagents and consumables were procured regularly when required.

Table 16 Inventory of infrastructure in Uganda, 2013–2017

Project item	Asset description	Serial no	Purpose	Status
1	Dell Computer OptiPlex 9010	8DN03W1	General office work	Working order
2	Dell Computer OptiPlex 9010	7JZKWX1	General office work	Working order
3	HP Envy Touch Smart 15 laptop	5CG3432XY2	General office work	Lost
4	HP Envy Touch Smart 15 laptop	5CG3433QGT	General office work	Lost
5	HP Envy Touch Smart 15 laptop	5CG3433TRD	General office work	Working order
6	HP LaserJet Printer	CNCXF7LOZP	Printing work	Working order
7	Insect Proof Screenhouse	Not available	Controlled experiments	Working order
8	Dell Computer OptiPlex 3020 MT	HF8S032	Accounts work	Working order
9	Plant Growth Chamber	AI15402701-6220V	Plant growth under controlled conditions	Working order
10	Campmaster Portable Freezer	45FYH13050126	Field sample collection, preservation and storage	Working order

SECTION THREE: Impacts, success stories and learning outcomes

Impacts

Impact area	Impact
How many students were trained by this project directly and indirectly?	419 students were trained: • 16 Diploma • 346 BSc • 52 MSc • 5 PhD.
No. of projects using the CDP facilities	Six projects at NaCRRI: • Mandi-Plus • GT4SP • African Whitefly Project • VIRCA-Plus • Double Haploid Project • ATAAS Other units at NaCRRI.
No. of students and/or staff using facilities and reagents of CDP	Twelve students and staff are using these facilities to run their experiments.
No. of people that have been inspired by the project	Some 750 people have been inspired by the project. These include most of the farmers trained, extension workers and some district leaders.
Institutional visibility	A number of tertiary institutions such as Makerere, Gulu and Nkumba Universities now apply to train their students at our facility. The National Fisheries Resources Research Institute generally use our ELISA plate reader for their work. The institute received 250 students in June 2018 from various institutes within the country as interns and volunteers. One student from Sierra Leone is being trained in molecular diagnostics. One student from Japan started an internship in July 2018. Twenty students, under the West African Virus Epidemiology project in West Africa, will come to NaCRRI in September 2018 for training on virus diagnostics and management.
Infrastructural capacity – helping student execute their project	Adequate infrastructure for studies such as efficient laboratory, research fields and a screenhouse.

	Two MSc students from Kyambogo University are doing their research at the facility with guidance from CDP staff.
New stakeholders interacting with the project	New stakeholders interacting with the project are OXFAM, A2N, UNFFE and BUCADEF.
Service the laboratory has provided	Diagnostics: 26,100 virus and vector samples Training: 422 scientist/students.
How many farmers have benefited either directly or indirectly	Some 5560 farmers have benefited. Directly: • 112 extension workers • 419 students • 681 farmers. Wider reach: Each trainee is assumed to have come from a household, and that each household comprises five people. This translates to 5560 people reached directly and indirectly by the project.
New collaborations/collaborative projects	Newly collaborated with "Cassava Applied Research for Food security in Northern Uganda" on demonstration gardens and farmer training, in which they invited us to train their farmers on the cassava value chain.
People using information generated by this project	Scientists: for cassava research work Students: for thesis write ups Policy makers: guide policy formulation Farmers: from the various information materials distributed and through training offered.
Benefits to the government – extension training, inspectors and regulators	Trained 112 extension workers and inspectors. These trainees work in various institutions such as Agricultural Research Stations and the Ministry of Agriculture across the country and their work includes guiding farmers in different communities.
Advocacy impacts on policy	The project advised the Uganda Government to put in place guidelines for certification and movement of planting materials across the country to limit the spread of CMD and CBSD.
Publications and other communications including other communication materials	Produced 19,200 communication materials and published two papers. These are being used by various communities and scientists.
Increase in crop yield and incomes (especially farmers who used clean materials)	Farmers' crop yields have increased due to knowledge gained and quality seed materials available to them for adoption.

Meetings and conferences attended – for the whole team	Three international conferences and two national meetings attended by project staff.
Support to breeders and other projects	Offered support to breeders in indexing their materials for viruses and diagnostics and this led to the release of new cassava varieties NAROCASS1 and NAROCASS2 in October 2016.
Involvement of vulnerable groups	Farmers are now selling cassava planting materials from stems obtained from the varieties grown in demonstration plots.
Change of farmers perceptions	Some members of the communities worked with vulnerable individuals such as the physically disabled, the elderly, youths, the poorly educated and those with limited access to land.
Other universities requesting to use the facilities/equipment	Farmers' perceptions of diseases in their crop changed; in particular, they saw the need to select clean planting materials and adopt new disease management strategies.
Minimizing/arresting brain drain	Most people trained by the project (i.e. staff, extension workers and crop inspectors) are working at various institutions within the country, hence contributing to the national economy and development.
Building a network of scientists	The project trained local and foreign scientists and collaborated with many local and foreign scientists. This resulted in a good network of knowledge. We continue to receive invitations to inspect private cassava farms for disease and training/guiding farmers on disease management.

Success stories

The reach of CDP in Uganda can be seen both within the country and abroad. Notable achievements are summarized below.

High profile at governmental level

NaCRRI released two varieties of cassava, one of which, NAROCASS1, is in very high demand in the country. The Uganda Government is procuring it and supplying it free to farmers under the program "Operation Wealth Creation" – a presidential initiative to create wealth through agriculture. Similarly, the Republic of Rwanda procured NAROCASS1 for distribution to its citizens.

Benefit to science and disease management

The project benefited Uganda by providing trained and qualified scientists within the country. One MSc and one PhD student were trained and have completed their studies successfully.

Trained scientists, extension workers and farmers can now deploy their knowledge and experience to work toward arresting the spread of cassava viral diseases in the country.

Benefit to farmers

The Genbadi Farmer Group was established in Pader district where a demonstration plot was set up in 2015. This group has been selling cassava planting materials to the Kitgum District Administration. This has enabled them to purchase one ox that they intend to use for plowing their land.

Most interacting farmers said they were willing to pay for disease-free planting materials because they have seen the benefits of such planting materials from colleagues within their communities.

Legacy of CDP

The long-term benefit of CDP is that farmers and other stakeholders are using the knowledge and seed materials given to them to improve production and productivity. This is shown by the increase in the area and percentage of improved cassava varieties grown by the various communities within the country.

Learning outcomes

This section summarizes events – whether advantageous or deleterious – which triggered a change from previous protocols, resulted in administrative changes or caused a task to be re-evaluated. It is intended to assist researchers at all levels when setting up or implementing similar projects in the future.

Value of project interaction with farmers

The demonstration gardens increased farmers' knowledge in general cassava production techniques with emphasis on pest and disease management to increase production and productivity. The seed multiplication plots increased farmers' access to high-quality cassava planting materials, which were lacking within the different communities.

This changed farmer practices, especially concerning the selection of planting sites, selection of good planting materials, planting time, spacing, size of stem cuttings to plant, removal of diseased plants within crop stands to remove virus inoculum source, mode of spread of the disease and farming as a business.

Value of project interaction with extension workers

The training of extension workers and crop inspectors improved the researcher–extension–farmer linkages in the country, which had previously been weakened due to lack of resources for training.

This revival enables extension workers to use their recently acquired knowledge within their communities. They can now assist farmers by inspecting fields for diseases as well as recommending sources of seed to potential buyers.

Improved knowledge of researchers on disease management

The surveys helped researchers to identify pest and disease hotspots in the country.

The knowledge has given researchers, breeders and pathologists the tools to determine where to deploy materials, where to test new varieties being developed, where to multiply seed materials and where to have farmers trained on disease management. This will assist with efforts to minimize disease spread.

Increased knowledge about the viruses and the vectors that spread cassava diseases

The characterization of viruses and disease vectors has improved researchers' knowledge on the species of viruses and pests infecting cassava in the country.

This is currently being used by researchers and students in experiments to determine the vector species that transmit particular virus species. This will guide future control interventions and the management of pests and diseases.

Refinement of procedures and protocols to aid similar tasks in future

The project improved on the techniques of sample collection, storage and analysis by developing sample collection techniques using the plant press method for CBSD laboratory materials. Samples used to be placed in ice – a method which led to the deterioration of samples prior to processing. The improved method has the advantage that researchers can maximize their time in the field for sample collection.

Importance of research collaboration

The project shared its data with valuable stakeholders locally and internationally and this has enhanced research collaborations with local and international partners – this can be seen in our collaboration with the West African Virus Epidemiology (WAVE) Project.

Enabling Uganda to assist other countries

Our experience on CDP now enables us to assist projects outside the country. For example, we are testing assorted West African cassava germplasm in our CBSD and CMD aggressive virus hotspots. This collaboration will help the WAVE regions to deal with potential outbreaks of CMD and CBSD.

Implications of our work at country level

The sharing of knowledge with collaborators enabled the Government of the Republic of Rwanda in 2016 to procure 11,000,000 pieces of cassava planting materials of varieties NAROCASS1 and NASE14 for their farmers. The variety NAROCASS1 was released as a result of giving conventional breeding support to breeders in Uganda by the project.

Forging new collaborations with NGOs

As a result of CDP, NaCRRI was approached by OXFAM, A2N and BUCADEF. NaCRRI was requested to assist with setting up farmer participatory demonstrations in their project areas to help farmers manage cassava viral diseases to increase yields.

Influencing administrative procedures at governmental level

The project's input has guided policy on the movement of cassava planting materials within the country. Plant materials are no longer allowed to move from disease hotspots to low disease pressure areas, but the reverse is possible. Moreover, materials must now have a movement permit proving that the source field was inspected by a competent authority and a certificate of quality issued.

Procurement procedures

Through their experience on CDP, researchers now know that they must plan their requirements in good time and to build in contingency to cope with delays or failure to receive materials ordered.

Effective presentation of our work in publications

Through joining the AgShare community, writing skills were improved and enabled the team to publish their work in well-regarded journals (see 'Publication of research findings'). Further manuscripts are due to be submitted for publication by the end of 2018.

Conclusions

The pest and disease surveys conducted by the NaCRRI team have determined the incidence, virus severity and whitefly species in the cassava-growing region of the country. Our studies have further shown that CMD and CBSD are the most important constraints to cassava production in Uganda.

Our work has directly benefited Uganda's cassava growers. The farmers gained knowledge on cassava production with an emphasis on pest and disease management. Additionally, they now have increased access to high-quality cassava varieties. Another important outcome is that researchers are now better informed about the hotspots of CMD and CBSD and this knowledge has guided the strategic deployment of materials by government and partner NGOs.

The high occurrence of improved varieties found in our surveys suggests that farmers have been able to obtain CMD-resistant and CBSD-tolerant cassava varieties. It also demonstrates that they appreciate the yields and level of resistance obtained from the improved varieties. The accepted use of improved varieties is the most important tool in the fight against viral diseases of cassava.

The CDP targets set at the start of the project were met. It is hoped that another phase of this work can take place to address the current gaps, such as limited market access by farmers for seed and the need to reach other communities with knowledge and quality seed.

Publication of research findings

Published by the project

Alicai, T., Ndunguru, J., Sseruwagi, P., Tairo, F., Okao-Okuja, G., Nanvubya, R., Kiiza, L., Kubatko, L., Kehoe, M. and Boykin, L. (2016). Cassava brown streak virus has a rapidly evolving genome: implications for virus speciation, variability, diagnosis and host resistance. *Nature Scientific Reports*, 6:36164.

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Mbanzibwa, D.R., Tian, Y.P., Tugume, A.K., Patil, B.L., Yadav, J.S., Bagewadi, B., Abarshi, M.M., Alicai, T., Changadeya, W., Mkumbira, J., Muli, M.B., Mukasa, S.B., Tairo, F., Baguma, Y., Kyamanywa, S., Kullaya, A., Maruthi, M.N., Fauquet, C.M. and Valkonen, J.P.T. (2011) Evolution of cassava brown streak-associated viruses. *Journal of General Virology*, 92:974–987.

Manuscripts in preparation

The following papers are expected to be submitted for publication by the end of 2018:

1. Prevalence of cassava brown streak disease and associated viruses in Uganda over ten years: 2008–2018
2. Prevalence of cassava mosaic disease and associated viruses in Uganda over ten years: 2008–2018
3. Experiences in promotion and adoption of virus-indexed planting materials in management of cassava virus diseases in Uganda
4. Evaluation of elite cassava progenitors for virus resistance in Uganda
5. Screening promising cassava germplasm for resistance to cassava brown streak disease by graft inoculation and field screening under high inoculum.

Acknowledgements

This work was supported by Tanzania Agricultural Research Institute (TARI)–Mikocheni with joint funding from the Bill & Melinda Gates Foundation (BMGF) and UK Department for International Development (DFID) through the 'Cassava disease diagnostics for sustainable productivity in Africa' project (Grant no. 51466).

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KENYA

Elijah Miinda Ateka[1], Justus Onguso[1], Samuel Kiarie Mwaura[1], Timothy Makori[1], Brenda Muga Akinyi[1], Martina Kyallo[2], Peter Sseruwagi[3], Fred Tairo[3] and Joseph Ndunguru[3]

[1] Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62,000 – 00200, Nairobi, Kenya
[2] Biosciences eastern and central Africa-International Livestock Research Institute (BecA-ILRI) Hub, P.O. Box 30709-00100, Nairobi, Kenya
[3] Tanzania Agricultural Research Institute (TARI)–Mikocheni, P.O. Box 6226, Dar es Salaam, Tanzania

Abstract

The Cassava Diagnostics Project (CDP) was implemented in Kenya from 2009 to 2017. The project aimed to (a) understand the threat from evolving viruses and vectors affecting cassava, (b) reach farmers directly and through partners and (c) build sustainable regional capacity. Cassava mosaic disease (CMD), cassava brown streak disease (CBSD) and whitefly abundance were monitored in country-wide surveys from 2009 to 2017. During these surveys in Kenya, 374 smallholder farms were visited throughout the cassava-growing areas of the country. Virus isolates and whitefly samples were collected from cassava and other plant species for laboratory analysis.

The survey results showed a decline in CMD incidence (from 53.7% to 39.5%) and CBSD incidence (from 25.8% to 11.5%) over the period of the surveys. The incidence of CMD and CBSD was highest in 2013 on the coast at 80.2% and 45.6%, respectively. Molecular characterization of cassava mosaic begomoviruses showed that *East African mosaic Cameroon virus* (EACMCV) was dominant in the Coast and Western regions and *East African cassava mosaic Zanzibar virus* was dominant in the Nyanza and Eastern regions. No new virus species were discovered but new begomovirus strains such as EACMCV were detected in 7.6% of the samples. This was the first time that EACMCV was detected in Kenya. Characterization of cassava brown streak viruses (CBSVs) revealed the presence of a DAG motif in all CBSVs, but not in the Ugandan cassava brown streak viruses, thus pointing to the possible transmission of CBSVs by aphids. Whitefly samples were characterized using *mitochondrial cytochrome I* gene markers that show the lineage and relatedness within and among *Bemisia tabaci* whitefly species. Phylogenetic analysis revealed that the SSA1 sub-clade I was geographically distributed in the Lake Victoria Basin agro-ecological zone but was completely absent along the coast. During our investigation of CBSVs, we evaluated the loop-mediated isothermic amplification procedure and found it to be specific and more sensitive than RT-PCR for detection of CBSVs.

The project extended support to conventional cassava breeders by confirming that their plants were tolerant or resistant to CMD and CBSD. Three varieties – Tajirika, Shibe and NI – showed promising resistance against CMD and CBSD, and were earmarked for further cassava improvement.

Our training programs were provided to 4489 farmers directly and to 84 extension officers. During these training sessions, the trainees learned about the causes, development, symptomatology, spread, diagnosis and management of cassava diseases. Our demonstration plots provided concrete evidence

of the benefits of clean planting materials as their yield was four times that of farmers' preferred varieties. An estimated 39,000 cassava cuttings, enough to establish 30 acres, were distributed to smallholder farmers from the demonstration plots. Four field days were conducted at the demonstration plots in Migori, Kilifi, Busia and Kitui counties.

The project enhanced the existing infrastructure of Jomo Kenyatta University of Agriculture and Technology through the procurement of laboratory equipment, greenhouses and a project vehicle. These facilities were made available to other university departments for student training. Outreach activities were conducted for farmers, extension staff, students, entrepreneurs and policy makers in seven demos, eight agricultural shows/exhibitions and four workshops. Various aspects of cassava production were addressed, and over 1500 brochures distributed. The project collectively enhanced the capacity of project staff through short courses and long-term training. As part of human resource capacity building, three MSc students and one PhD student successfully completed their research projects.

Acronyms and abbreviations

ACMV	*African cassava mosaic virus*
BLAST	Basic Local Alignment Search Tool
CBSD	Cassava brown streak disease
CBSV	*Cassava brown streak virus*
CDP	Cassava Diagnostics Project
CMB	Cassava mosaic begomoviruses
CMD	Cassava mosaic disease
DMV	*Denbollia mosaic virus*
EACMCV	*East African cassava mosaic Cameroon virus*
EACMV	*East African cassava mosaic virus*
EACMV-KE	*East African cassava mosaic virus*-Kenyan variant
EACMV-Ug	*East African cassava mosaic virus*-Ugandan variant
EACMZV	*East African cassava mosaic Zanzibar virus*
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KALRO	Kenya Agricultural and Livestock Research Organization
KEPHIS	Kenya Plant Health Inspectorate Service
LAMP	Loop-mediated isothermic amplification
MAP	Months after planting
RCA	Rolling-circle amplification
TARI	Tanzania Agricultural Research Institute
UCBSV	*Uganda cassava brown streak virus*
UWA	University of Western Australia
mtCOI	Mitochondrial cytochrome gene

Results summary: Kenya

Aim I: Understand the threat from evolving viruses and vectors

Objective 1: Disease epidemiology

Disease and whitefly prevalence surveys conducted	• Four surveys conducted: 2009 (94 fields), 2013 (112 fields), 2015 (141 fields) and 2017 (104 fields). • Mean cassava mosaic disease (CMD) incidence and severity index were as follows: 2009: incidence 48.3%; severity 3.4 2013: incidence 53.7%; severity 3.4 2015: incidence 50.4%; severity 3.7 2017: incidence 39.5%; severity 3.3. • Mean cassava brown streak disease (CBSD) incidence and severity index were as follows: 2009: not available at the time of writing 2013: incidence 25.8%; severity 2.6 2015: incidence 10.8 %; severity 3.0 2017: not available at the time of writing. • Mean whitefly populations per plant as follows: 2009: 4.2 2013: 1.3 2015: 1.0 2017: 2.2.
Alternative hosts for CBSVs and CMBs and associated insect vectors identified	• EACMV-UG2 was detected on one non-cassava plant, i.e. the bell bean tree (*Markhamia zanzibarica*) in Western Kenya (Kyallo et al., 2017).
Nature of interaction between CBSVs and CMBs and its impact on development of disease determined	• MSc student Brenda Muga found that the interaction of CBSVs and EACMV-Ug is specific at Ipomovirus and cultivar level. In two cultivars the expression of EACMV-Ug was significantly higher than when in co-infection with CBSV. The findings were submitted for publication in *Physiological and Molecular Plant Pathology*.

Objective 2: Characterization of emerging viruses

Cassava virus isolates in the project countries sequenced and analyzed	• Eight complete CBSV genomes published (Ateka et al., 2017). • An aphid-associated virus transmission DAG motif discovered in CBSVs and not UCBSVs. • There were 192 CMB partial sequences generated.
Cassava virus distribution maps for partner countries, generated (incidence, severity, whitefly)	• CMD and CBSD distribution and whitefly abundance maps for 2009 and 2015 were produced the using Microsoft PowerBI visualization tool.

Objective 3: Characterization of disease vectors

Whiteflies characterized	• There were 44 *mtCOI* sequences generated and published (Manani et al., 2017).

Aim II: Support clean seed systems for farmers	
Objective 6: Conventional breeding support	
Breeders' material monitored for disease and indexed for virus (CMBs and CBSVs) load at 3, 6, 9 and 12 months after planting (MAP)	• Three genotypes (NI, Shibe and Tajirika) that showed 0% incidence at 6 and 9 MAP promoted as part of the strategy to manage CMD. • Three genotypes (08/363, Shibe and Tajirika) with 0% incidence promoted as part of the strategy to manage CBSD.
Objective 9: Reaching farmers directly and through partners	
Farmers trained on CMD and CBSD disease symptom recognition and management strategies	• Six farmer groups or 4300 farmers trained at the demo sites, shows and during 2013, 2015 and 2017 disease surveillance surveys.
Demonstration plots for benefits of using virus-indexed planting materials established on-farm	• Seven demonstration sites were established, 300 farmers and 84 extension workers took part and were trained at the following locations: – 6 May 2014, Alupe – 7 November 2014, Homabay – 17 Feb 2015, Kitui – 4 May 2016, Funyula – 6 May 2016, Bungoma – 18 May 2016, Homabay – 22 May 2016, Kilifi.
Information materials developed and disseminated	• Over 1500 brochures on cassava production and viral disease identification and management were distributed; one radio program; 50 prevalence maps; and three newspaper articles. • Eight papers published in various peer-reviewed journals and three manuscripts are in preparation.
Aim III: Build sustainable regional capacity	
Objective 10: Strengthening stakeholder linkages	
Awareness on availability of diagnostic capacities created through training and different media	• One article on cassava viruses published in a national newspaper – The Daily Nation, *Cassava plant: the answer to food scarcity*, 20 September 2014, p.30. • One radio broadcast aired on topics relating to cassava virus diseases and management. • Two articles published in Jomo Kenyatta University of Agriculture and Technology (JKUAT) newsletter, Agritech News: – *Improved diagnostics capacity for cassava virus diseases* Issue 65 April–June 2016 vol. 54, p. 18 – *Hope for cassava farmers as JKUAT inaugurates diagnostics laboratory* Issue 67 Oct–Dec 2016 vol. 56. • One brochure on cassava production and viral disease identification and management developed (>1500 copies issued).

Objective 11: Strengthening human capacity and infrastructure	
Human capacity	
Project staff recruited	• Five project staff recruited: – Country team leader – Assistant country team leader – Research assistant – Lab technician – Driver.
PhD and MSc trained on different aspects of cassava virus diseases	• One PhD and one MSc student – both registered at Makerere University. Expected year of completion, 2018.
Advanced specialized training and visits for project scientists (1–2 months) conducted	• Dr Ateka, the country team leader, visited Rothamsted Research, UK, in 2015 for three months for training in disease modeling with the Cambridge disease modeling group. • Dr Ateka visited the University of Western Australia for three weeks for training on bioinformatics. The work resulting from this training was published in Ateka et al. (2017).
Extension workers, crop inspectors and other stakeholders (1 week) training	• There were 84 extension workers trained during 2014–2016: – 13 May 2014, Migori – 17–18 February 2015, Kitui – 10–11 March 2015, Busia – 25 November 2015, Kilifi – 17 August 2016, Homabay.
Project staff trained on IP, biosafety issues and communication strategies	• Dr Ateka attended training on these topics during 27–31 October 2014 at the International Centre for Research in Agroforestry.
Project results and information disseminated	• JKUAT CDP staff participated in agricultural shows and disseminated information on cassava viruses and the technologies to combat them.
Infrastructure strengthening	
Greenhouses constructed/renovated	• One new virus diagnostics laboratory and adjoining greenhouse completed, equipped and launched in 2016.
Project management	• Two accountants trained and provided with accounting package TALLY, 14–17 May 2013 in Dar es Salaam, Tanzania • Research assistant attended AgShare.Today training and scientific report writing skills in 2016 in San Diego, USA.

Background

Cassava (*Manihot esculanta* Cranz) is a major staple food and income generation crop in Kenya. Annual cassava production is 1,112,420 tonnes, the main production regions (Figure 1) being Coast (30%), Western (60%), Nyanza and Eastern (10%) (FAO, 2013). As the fifth most important food crop (after maize, wheat, potato and rice), cassava supports 30% of the estimated 40 million people in Kenya. Cassava is grown predominantly by small-scale farmers (Mwang'ombe et al., 2013). The cultivation of cassava in Kenya yields an average output of 5 t/ha, an amount substantially below Africa's average of 10.1 t/ha (FAO, 2013).

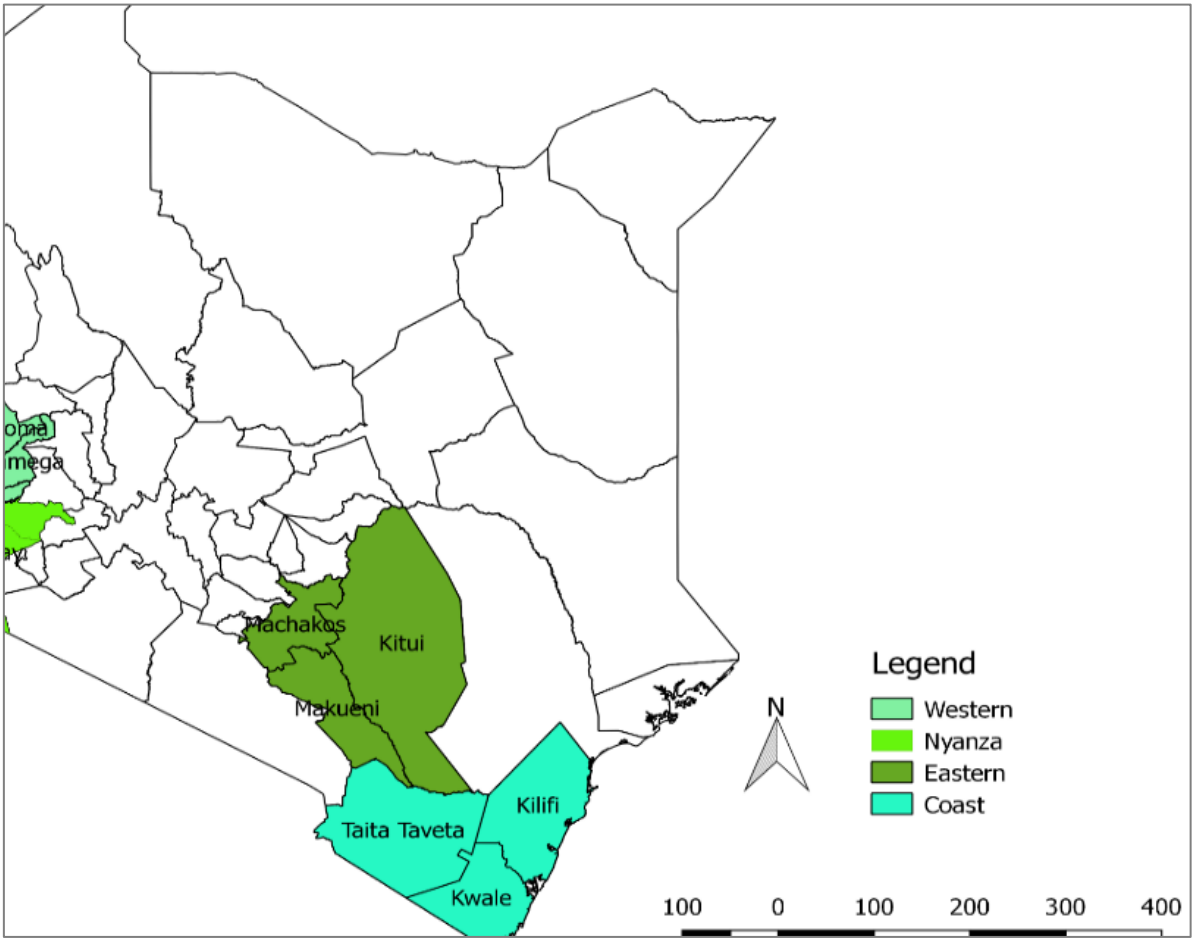


Figure 1 Cassava cultivation areas in Kenya

Cassava forms an important component of the cropping system in Kenya (Fermont et al., 2008). As in most parts of Africa, cassava in Kenya is a staple food. This important source of carbohydrates is also used as a raw material in industry and as feed for livestock (Hillocks and Jennings, 2003; Dixon et al., 2010; Rey and Vanderschuren, 2017). Under ideal growing conditions, disease-free cassava can be expected to yield more than 30 t/ha. However, despite its ability to thrive in difficult growing conditions, cassava output in Kenya does not meet that yield level. Major constraints to a healthy output include two major viral diseases: cassava mosaic disease (CMD) and cassava brown streak disease (CBSD).

The CMD is caused by eight ssDNA Cassava mosaic begomovirus (CMBs) in the genus *Begomovirus*, family *Geminiviridae*. These CMBs have been reported to cause CMD in Africa (Legg and Fauquet,

2004; Patil and Fauquet, 2009; Brown et al., 2015). The spread of CMD and its threat to cassava production in Kenya is attributed to the insect vector whitefly (*Bemisia tabaci*) and the movement of infected planting materials from one planting area to another. Symptoms attributable to CMD include misshapen leaves with a mosaic-like chlorosis and general plant stunting, and these lead to reduced tuberous root production. The CMBs comprise a number of individual virus species. Previous survey studies in Kenya identified *African cassava mosaic virus* (ACMV), *East African cassava mosaic Zanzibar virus* (EACMZV), *East African cassava mosaic Kenya virus* (EACMKV), as well as *East African cassava mosaic virus* (EACMV) and its variants: *East African cassava mosaic virus*-Ugandan variant (EACMV-Ug) and *East African cassava mosaic virus*-Kenyan Two variant (EACMV-KE2) (Bull et al., 2006; Winter et al., 2010).

Begomoviruses are an extremely successful group of emerging pathogenic viruses infecting cultivated (crops) and non-cultivated plants (Seal et al., 2006). Globally, wild plants from different botanical families have been identified as reservoir hosts for begomoviruses. The begomoviruses that thrive on uncultivated plants may emerge as new crop-infecting viruses, or may be involved in viral recombination, which in turn can lead to emergence of more virulent variants (Kyallo et al., 2017). Despite the potentially important epidemiological role played by weed-infecting begomoviruses in the epidemiology of crop diseases, these organisms remain understudied. Since East Africa is thought to be a center of diversity of CMBs, some effort has been expended to identify sources of new infections as well as their alternative hosts (Ndunguru et al., 2005).

Cassava brown streak disease (CBSD) is the second most important constraint affecting cassava production in Kenya after CMD (Were et al., 2007; Kathurima et al., 2016). It is a devastating disease that causes loss of root yield and quality. CBSD is caused by the cassava brown streak viruses namely *Cassava brown streak virus* (CBSV) and *Uganda cassava brown streak virus* (UCBSV); both these viruses belong to the genus *Ipomovirus* (family *Potyviridae*) (Winter et al., 2010). Symptoms of CBSD include brown lesions on the stem as well as veinal and general leaf chlorosis that may result in complete shoot die-back with severe disease conditions on tissues above ground. Mature stems show the streaks more strongly, especially under the bark of leaf scars (Hillocks and Jennings, 2003; Rey and Vanderschuren, 2017). Unlike CMD, the foliar symptoms are less conspicuous, and farmers are often unaware of the problem until the crop is harvested and the corky, yellow-brown necrosis in the root becomes evident (Were et al., 2007). In the starchy storage roots, symptoms appear as root surface fissures, constrictions and corky brown necrotic lesions that are largely responsible for yield losses of up to 70% in individual cassava fields. Damage from CBSD not only means that the tuberous roots do not fare well in storage but also renders them unsuitable for human consumption (Hillocks and Jennings, 2003). Prior to 2007, the distribution of CBSD was limited to the coast of Kenya but is now widespread in all areas where cassava is grown.

The Cassava Diagnostics Project (CDP) sought to build the capacity of Kenyan scientists to monitor the spread of the two diseases and the causal viruses in the key cassava production regions in Kenya. In addition, support was extended to cassava breeders to detect and quantify CMBs and CBSVs in advanced breeder materials, to improve the selection of resistant genotypes. Training and disease-awareness sessions were created for cassava farmers and extension agents: these sessions focused on the cause, effects, spread and management of CMD and CBSD. Details of the progress made and their impact are reported in the subsequent sections of the report.

SECTION ONE: Understanding the threat from evolving viruses and vectors

Disease epidemiology

Four surveys were carried out in the Coast, Nyanza, Western and Eastern regions of Kenya during 2009–2017. Field sampling and whitefly collection followed the protocol published in Sseruwagi et al. (2004). Results for the 2009 and 2015 surveys provide the comparative status of CMD, CBSD and their whitefly vector. Disease incidence from the 94 fields surveyed in 2009 is shown in Figure 2, and the status of these diseases among the 147 fields surveyed in 2015 is shown in Figure 3.

CMD and CBSD incidence

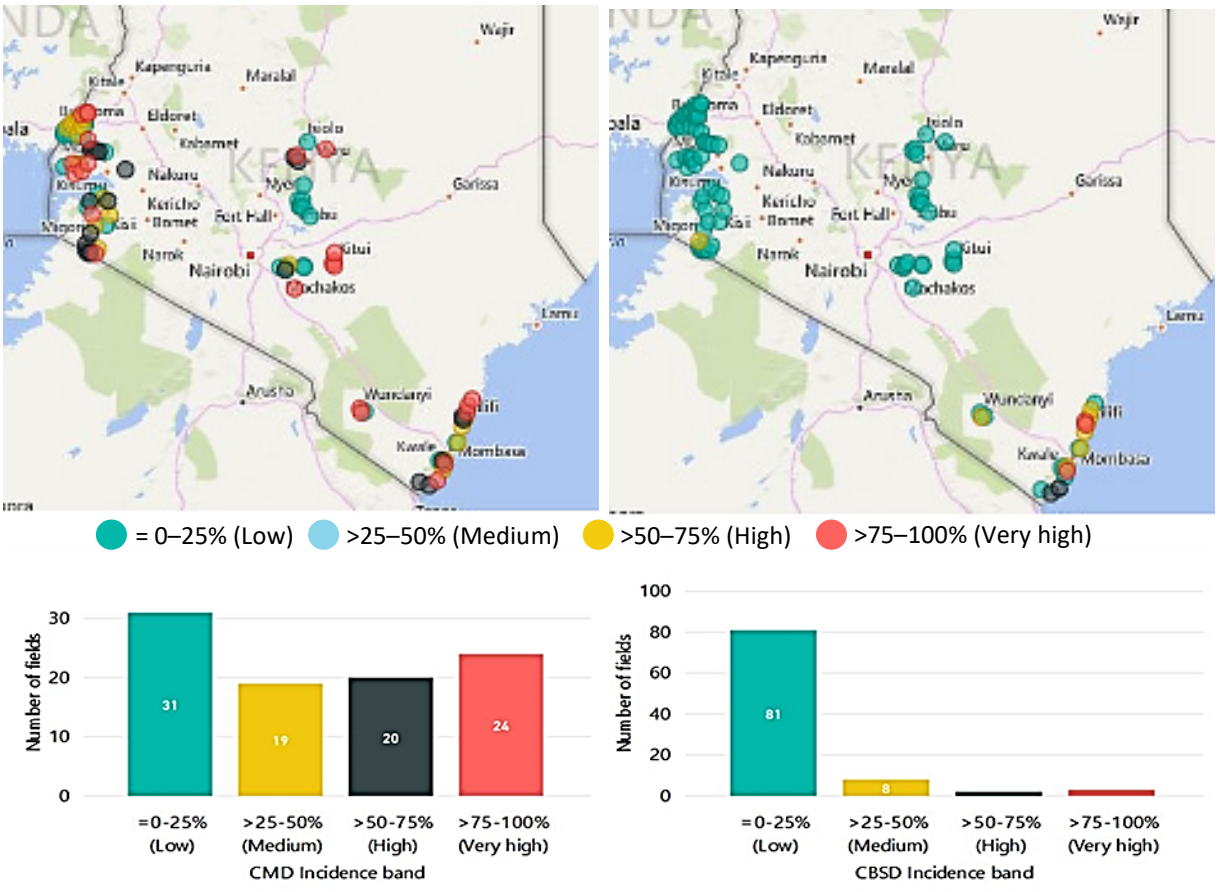


Figure 2 CMD (left) and CBSD (right) 2009 survey: locations sampled and calculated mean incidence at the locations

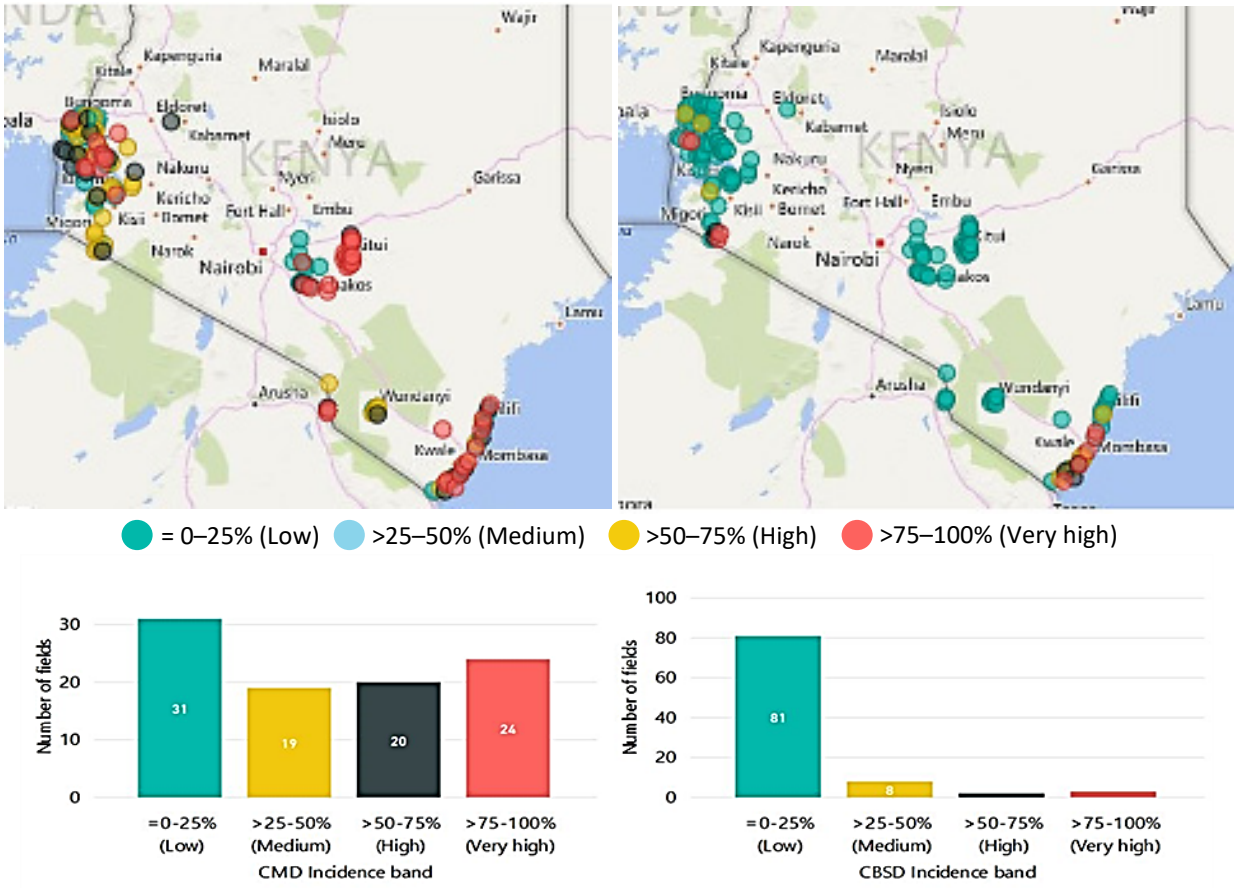


Figure 3 CMD (left) and CBSD (right) 2015 survey: locations sampled and calculated mean incidence at the locations

Although there was noticeable variation from one region to another, there were instances where the status of the disease remained unchanged – for example the Coast region where 60% incidence was recorded in both 2009 and 2015. Overall, however, CMD incidence declined from 2009 to 2015, and CBSD incidence increased slightly (Figure 4).

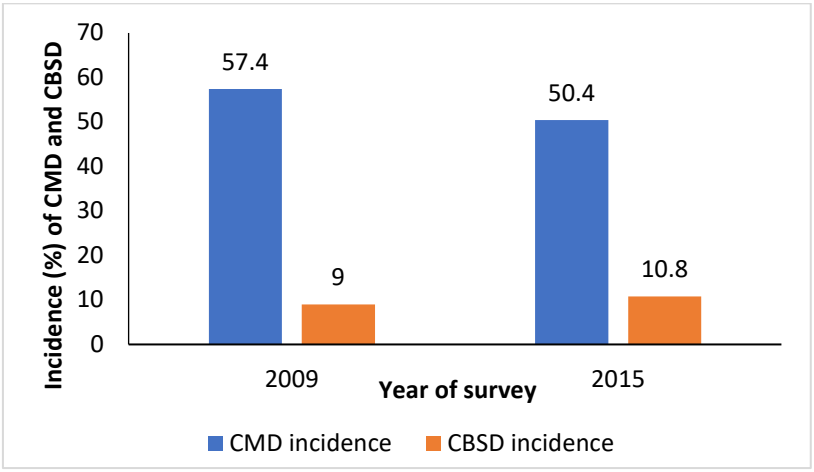


Figure 4 Mean CMD and CBSD incidence, 2009 and 2015

The whitefly population distribution based on our surveys is illustrated in Figure 5.

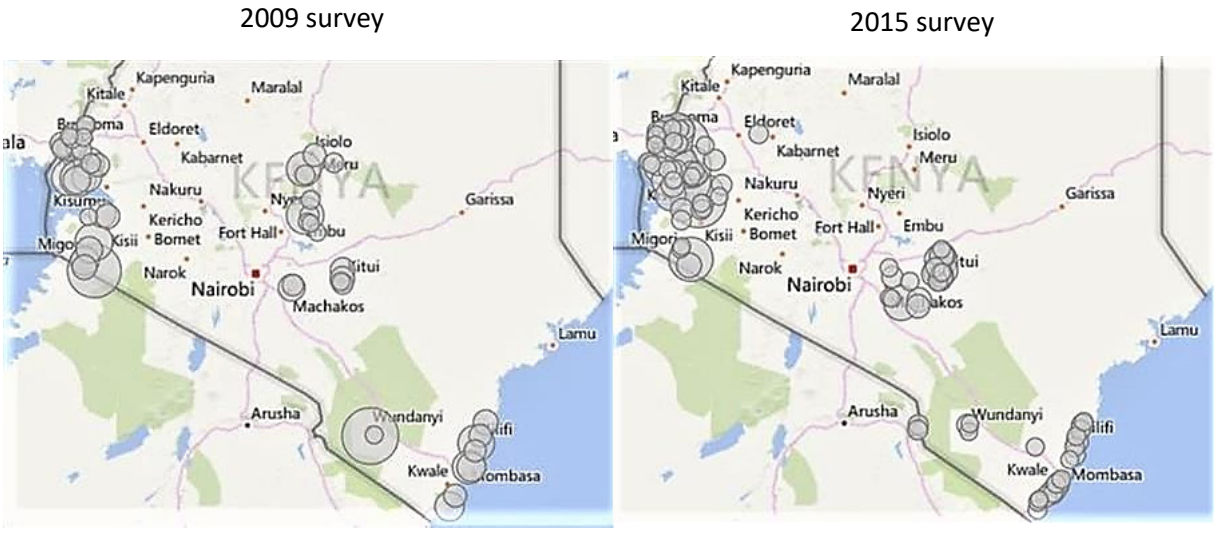


Figure 5 Distribution of whitefly population – based on locations surveyed. Grey circle sizes reflect the number of farms sampled

Analysis of the whitefly data indicated a decline in numbers in the locations surveyed (Figure 6). The count of adult whitefly per plant decreased from 4 in 2009 to 1 in 2015.

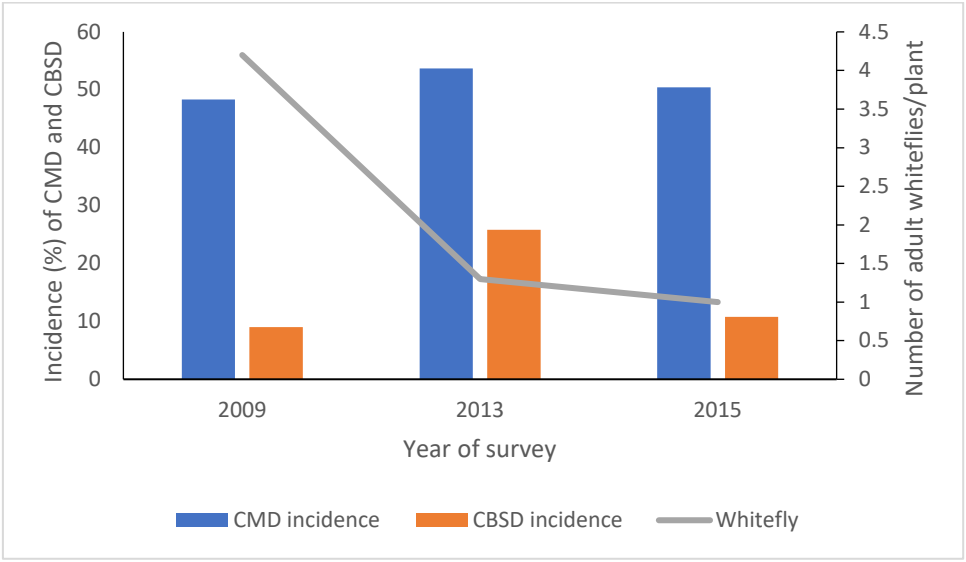
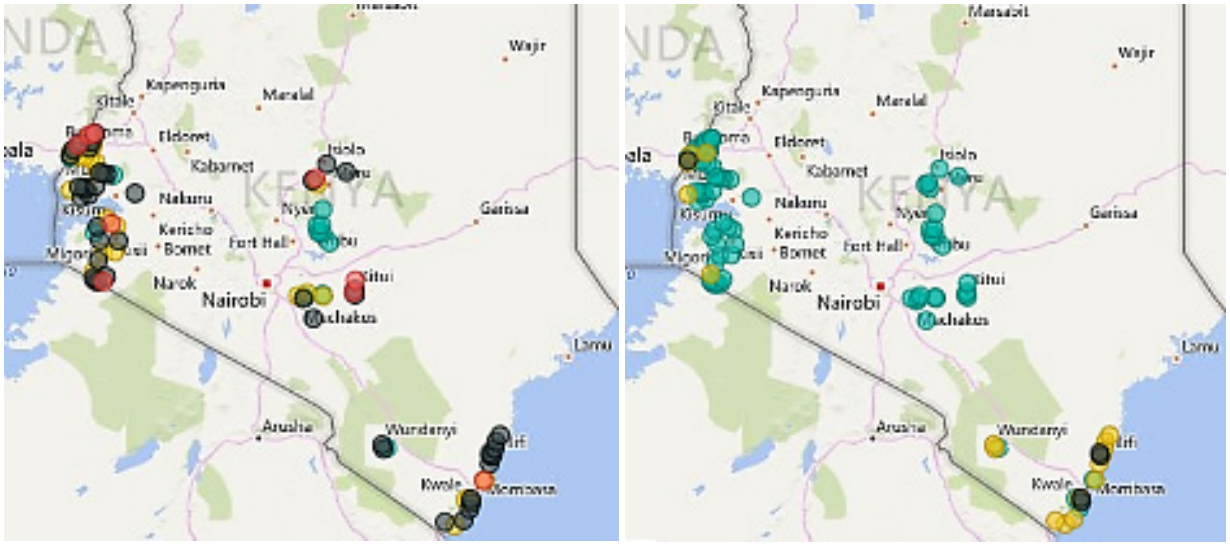


Figure 6 CMD, CBSD and whitefly abundance trends 2009–2015

Overall, our analysis showed a downward trend in disease incidence and whitefly population during our reporting period of 2009–2013. Factors that may account for this decline include (1) the severe drought in 2015 and (2) based on our work with farmers and extension workers, the increased disease awareness among farmers and their use of clean planting materials.

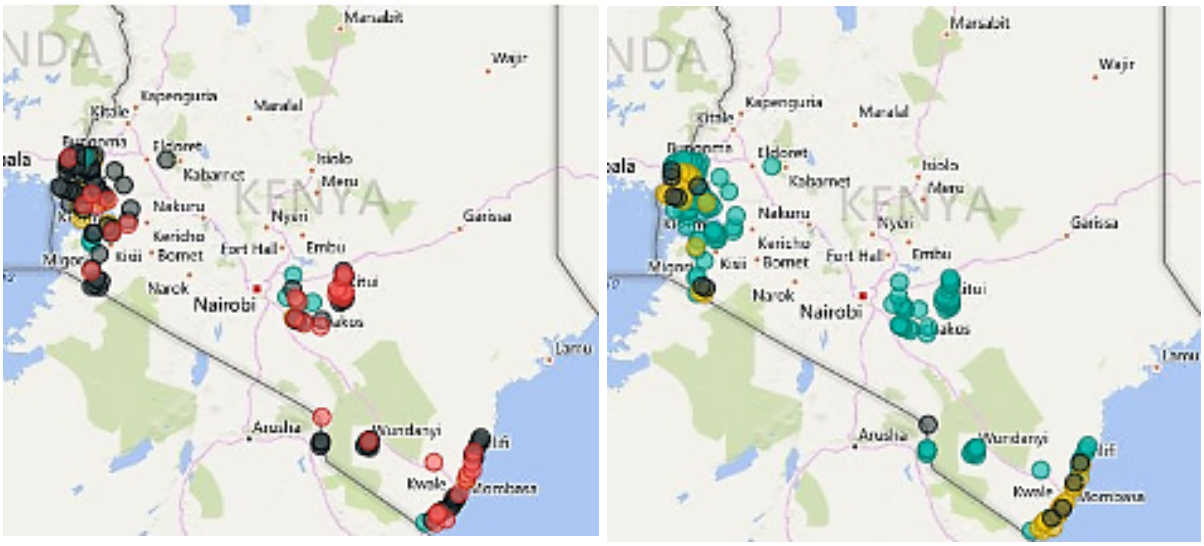
The recorded CMD and CBSD severity for the regions surveyed is shown in Figure 7 and Figure 8 for the same period.

CMD and CBSD severity



No infection Mild: = 2–3 Severe: >3–4 Very severe: >4–5

Figure 7 CMD (left) and CBSD (right) severity bands – 2009 survey



No infection Mild: = 2–3 Severe: >3–4 Very severe: >4–5

Figure 8 CMD (left) and CBSD (right) severity bands – 2015 survey

The graph in Figure 9 illustrates the disease severity trend during 2009–2015.

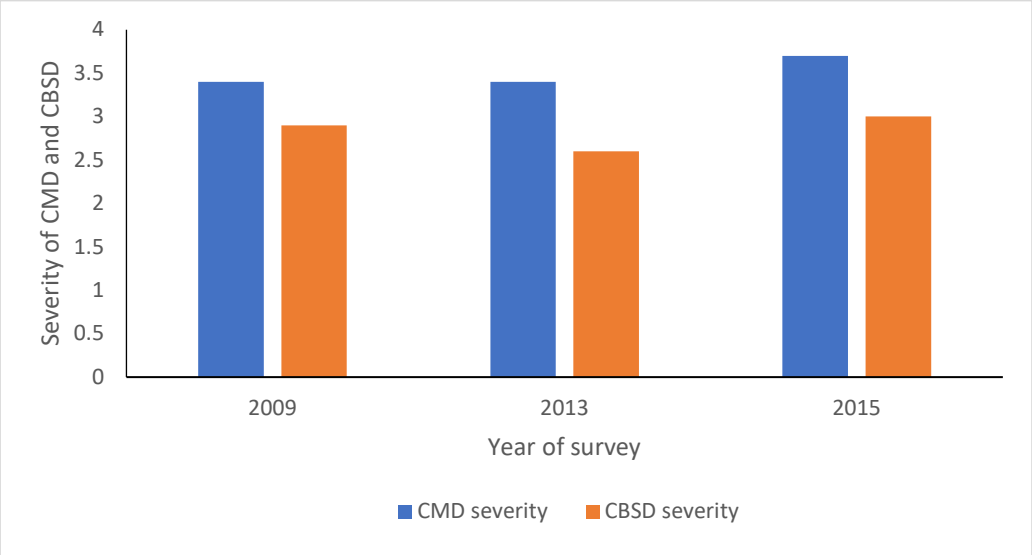


Figure 9 Mean CMD and CBSD severity trend 2009–2015

Detection of cassava-infecting viruses

CMBs

In the 2013 survey, samples were collected from plants showing CMD symptoms and tested with PCR using specific primers. The CMBs identified were EACMV, EACMKV, ACMV and EACMV-Ug. Based on the samples that tested positive for these virus strains, EACMV (59%) was the most widespread in the four regions followed by EACMV-Ug (23%) and ACMV (17%), with EACMV the least widespread (Table 1).

Table 1 Detection of cassava mosaic begomoviruses in samples from regions surveyed – 2013

		Virus strains			
Region	**No of samples**	**EACMV**	**EACMV-KE**	**ACMV**	**EACMV-Ug**
Western	70	48	18	20	31
Nyanza	83	30	0	11	11
Coast	50	35	2		11
Eastern	80	54	0	17	12
Total	**283**	**167** (59.0%)	**20** (7.1%)	**48** (17.0%)	**65** (23.0%)

Among the 251 samples collected during the 2015 survey, ACMV and EACMV-Ug were the most widespread among the regions surveyed (Table 2).

Table 2 Detection of cassava mosaic begomoviruses in samples from regions surveyed – 2015

		Virus strains			
Region	**No of samples**	**EACMV**	**EACMV-KE**	**ACMV**	**EACMV-Ug**
Western	59	39	0	39	23
Nyanza	60	33	28	39	21
Coast	70	27	0	21	25
Eastern	62	60	0	21	52
Total	**251**	**159** (63.3%)	**28** (11.1%)	**120** (47.8%)	**121** (48.2%)

CBSVs

Samples collected during the 2013 survey were tested for CBSVs using RT-PCR with virus-specific primers. Single and dual infections of CBSV and UCBSV were detected (Table 3).

Table 3 Detection of CBSVs in collected samples from regions surveyed – 2013

		Virus strains		
Region	**No of samples tested**	**UCBSV**	**CBSV**	**CBSV + UCBSV**
Western	78	18)	26	31
Nyanza	48	21	10	6
Coast	77	21	28	18
Eastern	0	0	0	0
Total	**203**	**60** (29.6%)	**64** (31.5%)	**55** (27.1%)

Similar findings were recorded from the 2015 survey (Table 4). Both CBSV and UCBSV were detected in Coast, Nyanza and Western regions of Kenya. Overall, UCBSV was more widespread.

Table 4 Detection of CBSVs in collected samples from regions surveyed – 2015

		Virus strains	
Region	**No of samples tested**	**CBSV**	**UCBSV**
Western	15	3	3
Eastern	0	0	0
Nyanza	16	8	0
Coast	45	14	31
Total	**76**	**25** (32.8%)	**51** (67.1%)

Genetic diversity of CMBs in Kenya

Diversity of CMBs in Kenya was determined through PCR, sequencing and phylogenetic analysis of the virus sequences. There was geographical clustering of EACMV and EACMV-Ug but not of EACMZV. EACMKV was found only in the Eastern region. For the first time, *East African cassava mosaic Cameroon virus* was identified but grouped separately from those found in West Africa with which they showed the highest similarity.

Genetic diversity of CBSVs

We set out to characterize the diversity of CBSVs in Kenya by determining whole genomes from 15 samples collected in the 2013 survey. Eight new CBSV whole genomes from Kenya were generated following RNA extraction, RT-PCR and next generation sequencing. The material that produced the complete genome sequences included three CBSV and five UCBSV samples. The sequences were analyzed using Geneious phylogenetic software (version R11.1) and were submitted to GenBank as accessions MG387652–MG387659. We discovered an aphid transmission-associated DAG motif within the coat protein of CBSV, but not in UCBSV in the Kenya sequences and all published complete and partial genomic sequences. The consistent presence of the DAG motif in CBSVs could be an area for future investigations, particularly the role of aphids in CBSD epidemiology.

Table 5 RT-PCR results of CBSV samples (from Ateka et al., 2017)

Sample ID	Region	Location	Geo-coordinates	Field ID	Variety	Infection-PCR results
K01	Western	Bumula	N00.59218, E034.49305	F6S1	Local	CBSV + UCBSV
K02	Western	Bumula	N00.399721, E034.44045	F7S3	Magana	CBSV
K04	Western	Teso	N00.55531, E034.16126	F14S3	Magana	UCBSV
K05	Western	Busia	N00.55531, E034.16126	F14S4	Magana	UCBSV + CBSV
K12	Coast	Malindi	S04.63214, E039.19356	F12S1	Local	USBSV + EACMV–Ke
K13	Coast	Msambweni	S04.65914, E039.20179	F13S1	Kibandameno	UCBSV + CBSV–Kilifi (Kenya)
K14	Coast	Lungalunga	S04.52837, E039.13629	F14S3	Kibandameno	CBSV + UCBSV, EACMV–Ke
K15	Coast	Lungalunga	S04.52826, E039.13598	F15S2	Kibandameno	CBSV

Bayesian analysis of the molecular data yielded a phylogenetic tree that placed the Kenya sequences within the CBSV clade. The new whole genomes from Kenya were found in every sub-clade of the CBSV phylogenetic tree (Figure 10). There are two major CBSV clades: CBSV and UCBSV. The K15 CBSV, from the Kenyan Coast (Lungalunga), was found in one clade with four other Tanzanian isolates. The remaining two CBSV genomes, K1 and K2, were samples from Western Kenya (Bumula). Five new Kenyan UCBSV whole genomes were added. Samples K4 and K5 formed a monophyletic group and were placed next to two Tanzanian genomes from Serengeti; K12 grouped with another Kenyan whole genome sequence (UCBSV_KE_54_FN433933); and K13 and K14 were on branches by themselves indicating new additions to the genetic diversity of CBSV phylogeny.

Interaction between CBSVs and CMBs and its impact on disease development

Co-infection of viruses occurs when two or more virus strains or species infect the same host at the same time. In some cases, co-infection results in a synergistic interaction in which the presence of one virus enhances the replication efficiency of the second virus and thus increases symptom expression (Wintermantel et al., 2005; Syller, 2012). Many viruses in the family *Potyviridae* are known to enhance the accumulation of at least some co-infecting viruses because the potyviral helper component proteinase (HC-pro) and/or P1 serine proteinase (P1) suppresses RNA-silencing mechanisms in the host plant (Stenger et al., 2007; Gil-Salas et al., 2012). The CBSVs (*Potyviridae*, Ipomovirus) have been reported to interact synergistically with CMBs (*Geminiviridae*, Begomovirus) in *Nicotiana benthamiana* (Irungu et al., 2010; Ogwok et al., 2010).

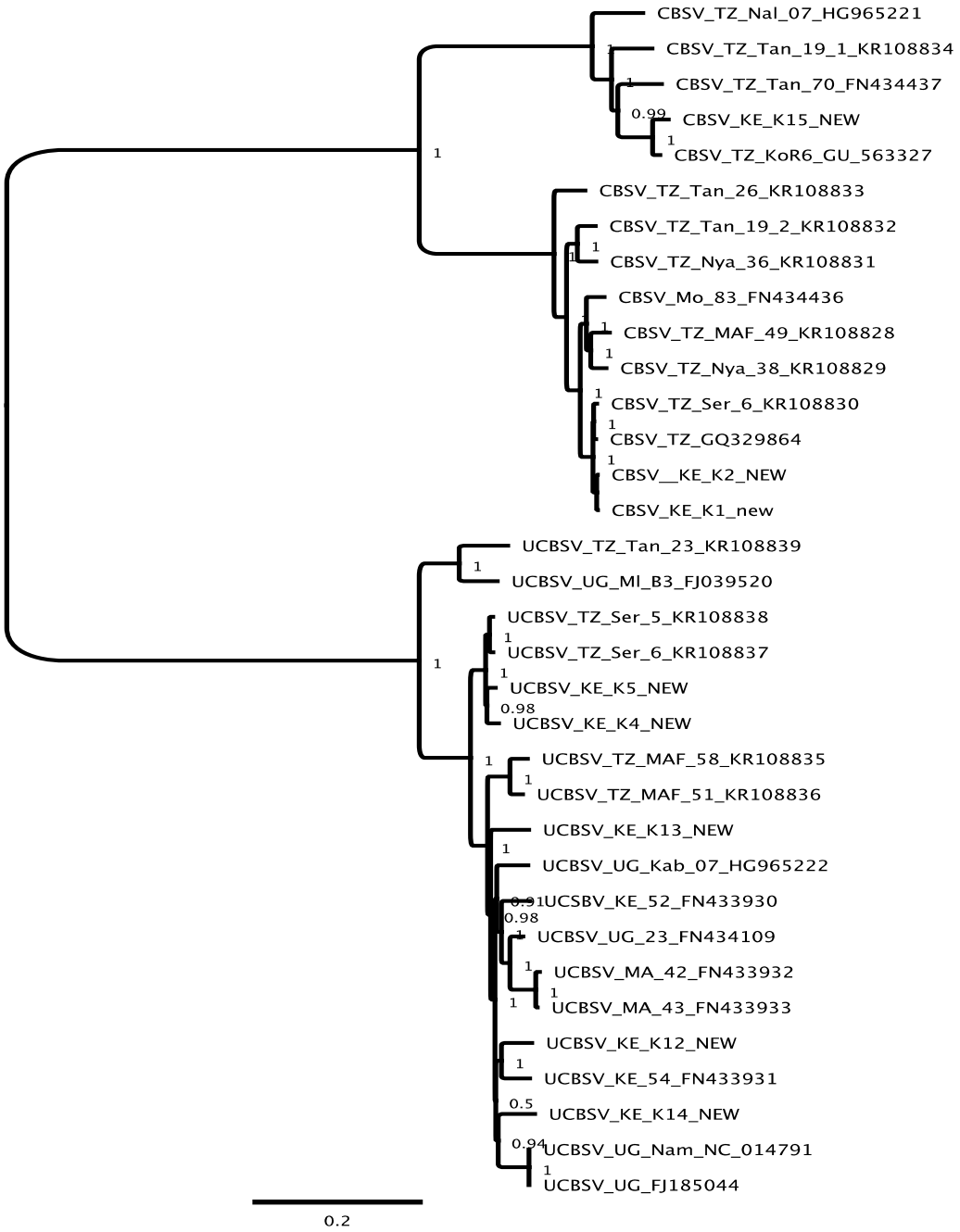


Figure 10 Phylogenetic tree of whole CBSV/UCBSV genomes (nucleotides) following Bayesian analyses using ExaBayes (adapted from Ateka et al., 2017)

The CDP Kenya MSc student, Ms Brenda Muga, investigated the effects of cassava co-infection of one begomovirus, EACMV-Ug, with two ipomoviruses (separately): CBSV and UCBSV. Compared with singly infected plants, EACMV-Ug viral titers were significantly lower in UCBSV co-infected plants of CMB-resistant variety MM 96/5280 at 3 months post inoculation and susceptible variety Mucericeri at 6 months post inoculation. In contrast, EACMV-Ug viral titer was significantly higher in CBSV co-infected than singly infected MM 96/5280 plants at 3 months post inoculation and Mucericeri plants at 6 months post inoculation. The interaction between EACMV-Ug and CBSVs was both cultivar-specific and ipomovirus-specific. Further work is needed to elucidate the pathogenesis and epidemiology of this interaction.

Determination of alternative hosts for CMBs and CBSVs

The soapberry shrub *Deinbollia borbonica* is a common weed in cassava fields in the coastal areas of Eastern Africa. Leaf samples with CMD-like symptoms were collected and screened with begomovirus degenerate and specific primers to confirm the presence of cassava-infecting viruses. A begomovirus, *Deinbollia mosaic virus* (DMV), with a highly conserved sequence was identified. Phylogenetic analysis grouped DMV together with crop-infecting begomoviruses including CMBs (Kyallo et al., 2017) (Figure 11A and Figure 11B). Transmission studies were conducted to confirm infection of cassava, which yielded negative results. The virus DMV was not found to infect cassava. Being a whitefly-transmitted virus, further biological characterization of DMV including recombination analysis is required to investigate any potential role in CMB diversity.

An additional study aimed at identifying non-cassava hosts of CMBs was conducted in Western Kenya. In May 2017, leaf samples were collected from symptomatic plants exhibiting virus-like symptoms ranging from mosaic, mottling, misshapen, twisted leaves and reduction in leaf surface area in plants belonging to the families *Solanaceae*, *Euphorbiaceae*, *Menispermaceae*, *Bignoniaceae* and *Meliaceae* found growing in three cassava fields in Busia and Homabay counties of Western Kenya. Asymptomatic plants from these plants were also collected.

From each family, total genomic DNA was extracted from three symptomatic and two asymptomatic leaves. Samples were tested with PCR using the diagnostic primer pairs JSP001/JSP002 for ACMV, JSP001/JSP003 for EACMV (Fondong et al., 2000), and UV AL1-F1/ACMV CP-R3 for EACMV-Ug (Zhou et al., 1997). A fragment of the expected size (1650 bp) was amplified with the primer pair UV AL1-F1/ACMV CP-R3 from symptomatic *Markhamia zanzibarica* samples collected from a cassava field in Homabay county. To confirm the PCR results, the genome of the virus was enriched using rolling-circle amplification (RCA) according to the protocol of TempliPhi 100 RCA Kit (GE Healthcare, USA) and deep sequenced using the Illumina MiSeq platform at Inqaba Biotech (South Africa). De novo assembly and BLASTn analysis (Altschul et al., 1990) identified two contigs as DNA-A and DNA-B belonging to EACMV.

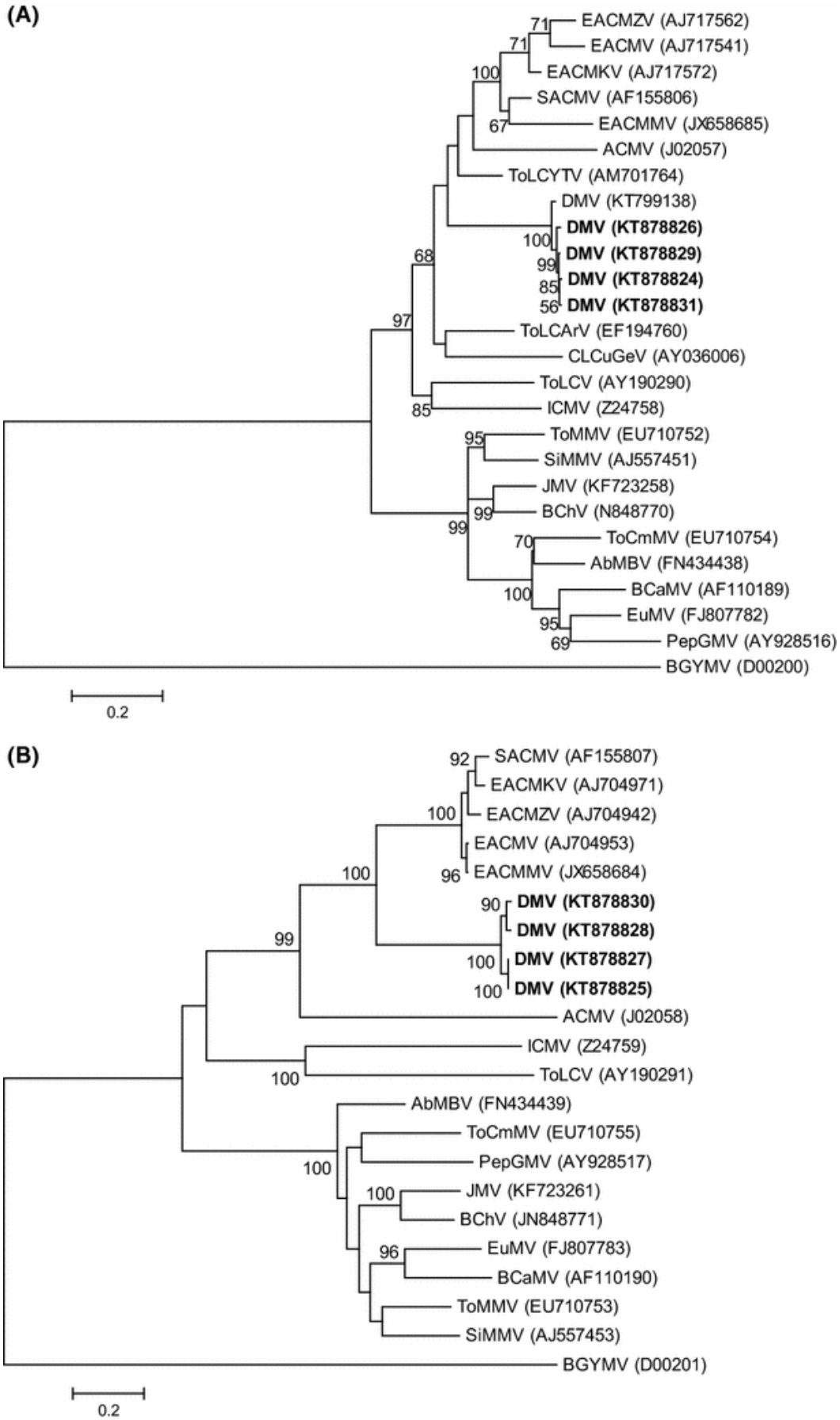


Figure 11 Maximum-likelihood trees inferred from the alignment of full-length DNA-A (A) and DNA-B (B) nucleotide sequences of DMV (in bold) and selected begomoviruses (adapted from Kyallo et al., 2017)

Characterization of disease vectors

Genetic diversity of *Bemisia tabaci* on cassava

At least 138 adult whiteflies were collected during the 2013 and 2015 surveys from major growing areas in Western, Nyanza, Eastern and Coast regions (Table 6). Primers were designed to amplify partial sequences of the *mitochondrial cytochrome oxidase I* (*mtCOI*) gene. The amplified *mtCOI* amplicons were sequenced (Table 6) and the sequences subjected to phylogenetic analysis using Bayesian analyses following multiple sequence alignment in Clustal W. Phylogenetic trees were then constructed to understand the genetic diversity across the study regions and were reported in Manani et al. (2017). Phylogenetic analysis revealed the presence of two distinct species: sub-Saharan Africa 1 (SSA1) comprising two clades (SSA1-SGI and SSA1-SGII) with percent sequence similarity ranging within 97.7–98.8%, and SSA2 with 99.5% (Manani et al., 2017). The SSA2 species associated with CMD were collected in the Western region bordering Uganda. The occurrence of SSA2 in Kenya was first reported 15 years ago (Legg et al., 2002).

Table 6 Number of whiteflies (by survey year) amplified and sequenced from various regions of Kenya

Survey	Samples amplified	Number of samples sequenced
2013	44	11
2015	94	33
Total	**138**	**44**

(Source: Manani et al., 2017)

The SSA1-SGI were geographically distributed along the lake basin climate area and absent along the coast; SSA1-SGII was concentrated along the coast and in Eastern and Nyanza regions. The SSA2 genetic group only occurred in the Western region neighboring Uganda.

Development of diagnostic tools

Development of a loop-mediated isothermal amplification assay CBSVs

Loop-mediated isothermic amplification (LAMP) is a technique that amplifies nucleic acids under isothermal conditions at temperatures of 60–65°C catalyzed by a DNA polymerase. LAMP assays have high specificity as amplification only occurs when six specific regions of the target amplicon are recognized by the primers. A PhD candidate, Mr Titus Kathurima, developed a CBSV reverse transcription LAMP (RT-LAMP) assay with a limit of detection at the dilution factor of 10^{-6} compared to 10^{-4} for RT-PCR. More work, however, is needed to make the procedure usable in the field.

SECTION TWO: Integrated pest management in Kenya

Conventional breeding support

Breeders' material monitored for disease and indexed for CMBs and CBSVs

The CDP Kenya team worked jointly with cassava breeders at the Kenya Agricultural and Livestock Research Organization (KALRO) in Mtwapa to set up a trial with seven elite genotypes (Table 7): 08/363, Shibe, Tajirika, Nl, F10-30-R2, Ex-Mariakani and Kibandameno. Incidence and severity were determined for both CMD and CBSD at 6 and 9 months after planting (MAP). PCR detection of CMBs and CBSVs was done for the seven varieties at each assessment. The whitefly populations were determined for each test variety at 3, 6 and 9 MAP.

Figure 12 Project team visiting breeders' material at KALRO Mtwapa

Incidence and severity of CMD and whitefly populations

The mean CMD incidence varied significantly among the genotypes. The most common symptoms were mottling, mosaic and vein banding, leaf chlorosis, shoe string and distortion particularly on Kibandameno variety. At 6 and 9 MAP, Kibandameno showed the highest mean incidence at 74.97 and 82.47%, respectively. Varieties NI, Shibe and Tajirika had no CMD symptoms at 9 MAP. The respective CMD severities of the seven varieties at 6 and 9 MAP are shown in Table 7.

Table 7 Incidence and severity of CMD and whitefly populations in breeder cassava genotypes at 6 and 9 MAP, Mtwapa, Kenya 2013

Genotype	6 MAP			9 MAP		
	Incidence %	Mean severity index	Mean whitefly count	Incidence %	Mean severity index	Mean whitefly count
F10-30-R2	0a	1	1.81	29.99	2.10	1.47
Nl	0a	1	2.57	0	1	2.68
Shibe	0a	1	1.14	0	1	1.14
Tajirika	0a	1	1.59	0	1	0.89
08/363	7.5	2.03	1.31	26.24	2.13	1.31
Ex-Kibandame	26.24	2.18	0.68	26.24	2.18	0.68
	74.97	4.59	2.39	82.47	4.66	2.25

Significant varietal ($P < 0.001$) differences in whitefly infestation were observed among the varieties. At 6 MAP, the highest number of whiteflies (2.568) were recorded on Nl. At 9 MAP, Nl still recorded the highest mean number of whiteflies (2.682). This was followed by Kibandameno and F10-30-R2 with a mean number of 2.246 and 1.474 whiteflies per five top-most leaves, respectively. Ex-Mariakani exhibited the least number of whiteflies with a mean of 0.68.

Incidence and severity of CBSD

Incidence of CBSD significantly ($P < 0.001$) differed among the genotypes, with ranges of 0–75% and 0–89% at 6 and 9 MAP, respectively (Table 8). Varieties had foliar symptoms indicating CBSD, with the exception of Tajirika, NI and Shibe. Incidence was highest (75%) in Kibandameno and Ex-Mariakani at the different times of the crop cycle (Table 8). In most varieties, the severity increased with plant age. The lowest severities (of 1) were recorded in varieties Tajirika, Shibe and 08/363. Both CBSV and UCBSV were detected from the breeder genotypes used in this study (Table 8).

Table 8 Incidence and severity of CBSD in breeder cassava genotypes at 6 and 9 MAP, Mtwapa, Kenya in 2013

Genotype	6 MAP		9 MAP	
	Incidence (%)	Severity	Incidence (%)	Severity
Shibe	0	1	0	1
Tajirika	0	1	0	1
08/363	7.5	1	26.2	1
F10-30-R2	0	1.2	29.9	1.2
Nl	0	1.4	0.0	1.4
Ex-Mariakani	26.2	1.3	26.2	1.3
Kibandameno	74.9	1.5	89.9	1.5

Reaching farmers directly and through partners

Awareness creation and training on CMD and CBSD symptom recognition and management strategies

One of the most effective ways of managing cassava viral diseases is the use of clean planting materials. However, the success of this strategy depends on the knowledge and awareness of key players such as farmers and crop extension officers. Farmers need to be conversant with CMD and CBSD symptoms and the management options available to combat these diseases. Awareness campaigns were focused on the symptoms and management options available, especially the use of virus-free planting materials.

Field training for disease surveillance surveys

During the field surveys, the owners of the sampled cassava fields (374 farmers) were given one-to-one training on CMD and CBSD symptom recognition and management (Figure 13).

Figure 13 Farmers being trained by CDP team on symptom identification and management during surveys

Training at agricultural shows and exhibitions

The CDP Kenya team participated in 10 agricultural shows during 2014–2016; for instance, the team participated in the Nyeri, Migori and Nairobi agricultural shows each year. Some 4900 people visited the Jomo Kenyatta University of Agriculture and Technology (JKUAT) stand that housed the CDP stand. Visitors included farmers, primary, secondary and university students and staff, researchers, business owners and their staff, entrepreneurs, policy makers and the general public. These are summarized below (Table 9). The key messages were on disease symptom recognition and management options for CMD and CBSD. During the shows and exhibitions, brochures were given out covering various aspects of virus diseases infecting cassava, their management and cassava utilization. Additionally, the project participated in the JKUAT scientific conference in 2015 and 2016 where we exhibited material relating to CMD and CBSD and their management strategies. Some 200 brochures were issued, covering various aspects of both diseases and their management.

Table 9 Number and categories of visitors at public events

Year	Farmer visitors	Business visitors	Researchers	Extension officers	Schools/ universities reached	Policy makers	Total
2013	1829	1	22	46	79	11	1988
2014	633	6	19	31	21	6	716
2015	1192	5	25	16	44	8	1290
2016	835	4	19	7	74	3	942

Figure 14 H.E. Uhuru Kenyatta President of Kenya at Nyeri ASK Show (left) and project team describes virus symptoms to show attendees at Nairobi international trade fair in 2016

Figure 15 CDP Kenya team interacts with farmers during a field day at Kitui Farmers Training Center in 2013

Cultivating demonstration plots to underline the benefits of using virus-indexed plant material

One of the successful methods of passing messages to farmers is the use of practical methods – i.e. demonstration plots. This strategy was aimed at building critical knowledge among farmers so that they could recognize and sustainably manage CMD and CBSD. Observing the benefits of using clean planting materials usually leads to higher adoption rates of a technology or techniques. This activity was also used to demonstrate good cultivation practices. Demonstration plots of 10 m^2 were established according to the CDP harmonized protocol using disease-free planting material (Sseruwagi et al., 2004). These were adjacent to farmer-managed plots which were planted with the farmers' preferred variety. Additionally, a one-acre plot was planted with an improved cultivar (MH96/0186) for seed multiplication.

Seven demonstration plots and seven virus-free cassava multiplication plots were established in Coast, Western, Eastern and Nyanza (Kehancha) regions (Table 10). In each location, the selection of demo sites was done with the help of County Agricultural Officers who participated in the activity as collaborators. A total of 1000 farmers from different farmer groups participated in the demonstration plot experiments. In general, farmer groups were selected according to their location, gender and needs. A farmer group was chosen if cassava was one of its focus crops. Women formed approximately 60% of the farmers within each group comprising 30–50 members.

Distribution of cuttings to the farmers in the group, and others in the vicinity of the demonstration plots, followed a multiplication and distribution strategy that recorded the recipients of the materials, and tasked them to give back an equal amount of the materials to the project for further distribution (Figure 16). Training was conducted at the demonstration plot location, at intervals of 3, 6, 9 and 12 MAP. The CDP team made regular field visits to monitor the trials.

Table 10 Location of CDP demonstration plots and field activities in Kenya

Site/ Institution	Province	District	Latitude	Longitude	Activity
KARI-Alupe	Western	Teso	0.33906	34.3651	Multiplication, Open quarantine, Germplasm
Kilifi	Coast	Kilifi	−3.86207	39.74467	Multiplication, Open quarantine, Germplasm
Kehancha	Nyanza	Migori	−1.1601	34.62315	Multiplication, Open quarantine, Germplasm
Kitui	Eastern	Kitui	1.3745	38.01001	Multiplication, Open quarantine, Germplasm
Kimaeti	Western	Bungoma	0.64347	34.50793	Multiplication, Open quarantine, Germplasm
Funyula	Western	Busia	0.5622	34.17064	Multiplication, Open quarantine, Germplasm
Homabay	Nyanza	Homabay	−0.42202	34.78882	Multiplication, Open quarantine, Germplasm

Figure 16 Farmers prepare plots (left) and select preferred materials (right) for planting, Bungoma county, 2016

Figure 17 Virus-free seed multiplication plot (JKUAT, 2015)

In the Coast region, cassava root yield from virus-free cultivars in demonstration plots had high average weight (9.8 kg) compared to the farmers' preferred variety (MH96/0186) with 2.47 kg. Busia and Migori had similar results, except with different local farmer-preferred varieties. The yields of the crop established using clean seed was four times that of the field managed by farmers (which used untested seed). Approximately 39,800 virus-free cuttings were distributed to farmers in the four regions (Table 11).

Table 11 Number of cuttings distributed, and farmers reached 2015–2017

Region	Number of cuttings	Farmers reached
Kilifi	6000	280
Migori	3000	103
Busia	5300	109
Kitui	2000	35
Homabay	1500	50
Bungoma	2000	55
JKUAT	20,000	33
Total	**39,800**	**665**

Figure 18 Farmers show harvests obtained from cultivars Karembo and Kibandameno (left) and a farmer admires cultivar Karembo (right), in Kilifi county, 2015

Figure 19 Farmers receiving cuttings from demonstration farms

Information materials developed and disseminated

Communications – creating awareness

Awareness materials were developed and disseminated on CMD and CBSD management and the benefits of using virus-free planting materials. These materials included articles in newspapers and/or magazines, radio interviews, brochures and disease prevalence maps (Table 12). A key article "Seeds of Gold" on cassava diseases was published in *The Nation* in September 2014. Other articles with the same message were published in the JKUAT magazine *Agritech News* (2016a, 2016b). Additionally, a one-hour audio interview was carried out for Radio Waumini, which has a country-wide audience.

Table 12 Information material on cassava viral diseases and their management for different target groups 2014–2017

S/No.	Material	Topic	Target audience	Quantity issued
1	Brochure	Recognition of CMD and CBSD and management	Farmers, extension agents	>1500
2	Radio messages	About CDP, importance of cassava diseases and how to manage them	Farmers, extension agents	1
3	Newspaper article	About CDP, importance of cassava diseases and how to manage them	Farmers, extension agents, researchers	2
4	Prevalence maps	Distribution and incidence and severity of CMD in Kenya	Breeders, virologists	50

Reaching farmers indirectly through partners

Farmers were indirectly reached through the crop extension officers trained during the training of trainers (Figure 20 and Figure 21). A total of 84 crop officers were trained during five training events in Kitui, Busia, Bungoma, Homabay and Migori counties of Kenya in 2014–2015 (Table 13). Ten county crop extension officers were also trained in a field day held in Kilifi in the Coast region in collaboration with the County Agricultural Office. It is expected that these officers will, in turn, train farmers and colleagues in the course of their extension duties.

Table 13 Number of extension officers trained in different counties

Region	County	Number of officers trained
Eastern	Kitui	18
Nyanza	Migori	10
Western	Busia	16
Western	Bungoma	20
Nyanza	Homabay	20
TOTAL		**84**

Figure 20 Desk-based and field training at Migori, Nyanza region

Figure 21 Desk-based training at Eastern and Western regions respectively

Outputs from the training

- Trainees were able to identify and distinguish CMD from CBSD symptoms. They were also able to identify mite damage
- Trainees were able to differentiate between whitefly- and cutting-borne infections
- Crop extension officers expressed willingness to work with researchers in reaching out to growers
- The crop extension officers agreed to sensitize farmers and seed merchants to use virus-tested materials because their virus status was known
- Crop extension officers requested that more demonstration plots be established.

Build sustainable regional capacity

Strengthening stakeholder linkages

Stakeholder engagement

A number of stakeholders were approached and contact maintained throughout the project. These interested parties ranged from University departments to research institutions and to government departments (see Table 14).

Table 14 Partners and stakeholders visited during the impact assessment baseline study and monitoring and evaluation missions for the 'Disease diagnostics for sustainable cassava productivity in Africa' project, 2014

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
Jomo Kenyatta University of Agriculture and Technology (JKUAT)	Thika Road, Nairobi	University (Project partner)	Dr Elijah Ateka (Project Country Team Leader), Samuel Mwaura (Research Assistant), Timothy Makori (Technician)	Project management, training, supervision of students and research	Dr Elijah Ateka and Mr Samuel Mwaura
		University (Non-project staff)	Mr Francis Ombwara (Chief Technologist)	Working on disease diagnostics	Dr Elijah Ateka
Kenya Plant Health and Inspection Services (KEPHIS)	Njoro, Nairobi	Government	Francis Mwatuni, (Officer in Charge, Plant quarantine and bio-security)	Plant health, seed certification & bio-security	Mr Francis Mwatuni
Kenya Agricultural Research Institute (KARI), Mtwapa	Mtwapa, Mombasa	NARS	Dr Theresia L. Munga	Breeding for resistance to CMD and CBSD	Dr Theresia L. Munga
		National Agricultural Research Systems (NARS)	Mr Dau Mwakina, (Technician)	Disease resistance CMD and CBSD	Mr Dau Mwakina
Kenya Agricultural Research Institute (KARI), Njoro	Njoro, Nakuru	NARS	Dr Laura Shali Karanja (Principle Research Officer), Mr Henry Okwaro (Research Officer) Mr. John Ndungu,	Production of disease-free planting materials using tissue culture, Disease diagnostics and quality control	Dr Laura Shali Karanja and Mr John Ndungu

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
			(Quality control Officer)		
Biosciences for East and Central Africa (BecA)	Nairobi	International Agricultural Research Institute (Collaborator)	Dr Rob Skilton (Team Leader Capacity Building)	Capacity building and biosciences services (sequencing, training, student supervision)	Dr Rob Skilton
		Project student	Ms Martina Kyallo (PhD student)	Research study	Ms Martina Kyallo
University of Nairobi (UoN)	Nairobi	University (Collaborator)	Dr Douglas Miano (Lecturer and supervisor)	Training and student supervision	Dr Douglas Miano
GTIL, Nairobi	Nairobi	Private tissue culture laboratory	Ms. Judith Kilonzo (Laboratory Manager)	Production of disease-free planting materials using tissue culture	Ms Judith Kilonzo
Farmers, Mombasa	Mombasa	Farmer	Mr Emmanuel Mbotela and Ms. Salome Nyambura	Cassava production	Mr Emmanuel Mbotela

Eighty-four extension officers from the Ministry of Agriculture and KALRO were trained on disease identification and management by JKUAT personnel in the cassava-growing regions.

Exchange visits between scientists in CDP project countries were established. A scientific meeting was held in Zambia in May 2016 and was attended by the CDP country team leaders. The objective was to exchange views and ideas on project activities. The visit included participants from Tanzania, Malawi, Kenya, Rwanda, Uganda and Mozambique. During that event, a field visit was made to a farmer's field in Rufunsa and the team met with local farmers as well as visiting the University of Zambia. During both visits, country team leaders, researchers and farmers were able to share knowledge, ideas and experiences about their work on cassava.

Strengthening human capacity and infrastructure

Human capacity

Several training events were undertaken by various members of CDP Kenya. These included short and long courses (PhD and MSc programs), seminars, workshops and visits to advanced laboratories. The scope of training ranged from non-scientific domains (e.g. leadership skills, financial management and accounting) to more technical spheres (e.g. bioinformatics, modeling and intellectual property management). Table 15 lists the short courses held during 2013–2017.

Table 15 Training events for CDP Kenya staff and students, 2013–2017

Dates	Course title	Venue	Trained
13–17 May 2013	Financial Management and Accounting Package	Dar es Salaam	Elijah Ateka, Esther Muthoni, Juma M.
November 2013	Leadership and Management Skills	Entebbe	Elijah Ateka
27–31 October 2014	Intellectual Property Rights	Nairobi	Elijah Ateka
17–19 February 2015	Data Management Training	Kunduchi Beach Hotel	Elijah Ateka
May 2015	Modeling of Cassava Virus Diseases and Development of Disease Prevalence Maps	Rothamsted Research and Univ. of Cambridge	Elijah Ateka
January 2016	Sequence Analysis of Kenyan CBSVs	University of Western Australia (UWA)	Elijah Ateka
February 2016	Bioinformatics	Dar es Salaam	Timothy Makori, Brenda Muga
June 2016	Bioinformatics and Whitefly Species Identification	JKUAT	Over 20 Kenyan students, three Zambian scientists
October 2016	Scientific Writing	Lusaka	Brenda Muga
December 2016	Data Entry and Assembly	Dar es Salaam	Samuel Mwaura, Brenda Muga

PhD and MSc students trained on different aspects of cassava virus diseases

One MSc student (Brenda Muga) and one PhD student (Martina Kyallo) were fully supported by the CDP. In addition, two MSc students (Geoffrey Singombe and Duke Manani) were supported with their research work by the project. All four students successfully completed their research work (Table 16).

Table 16 Postgraduate students supported by CDP – Kenya

S/No	Name and program	Nature of support	University	Research topic
1	Martina Kyallo (PhD)	Full	Makerere	Characterization of begomoviruses infecting non-cassava host plants in East Africa
2	Brenda Muga (MSc)	Full	Makerere	Effect of co-infection of cassava mosaic begomoviruses and cassava brown streak viruses on virus accumulation
3	Duke Manani (MSc)	Partial	JKUAT	Characterization of Kenyan whitefly *Bemisia tabaci* using the *mtCOI* gene
4	Geoffrey Singombe (MSc)	Partial	JKUAT	Evaluation of Kenyan breeder materials for viruses infecting cassava

Staff training in advanced labs

Training in disease epidemiology and modeling

Dr Elijah Ateka visited Rothamsted Research and Cambridge University, UK, to work with the Modeling Group on modeling of cassava virus disease spread and epidemiology using the data collected by the CDP country surveys in the seven countries. During the visit, the scientist in collaboration with counterparts at Cambridge and Rothamsted (Figure 22) and Dr Patrick Chikoti, the country team leader of Zambia in the CDP, developed a strategy for modeling, which helped the teams better understand the spread of CMD and CBSD in complex cropping systems in sub-Saharan Africa.

Figure 22 Collaboration between CDP Zambia, Rothamsted, Cambridge University and CDP JKUAT. From left to right: David Godding, Patrick Chiza Chikoti, Elijah Ateka, Chris Gilligan and Anna Szyniszewska

Bioinformatics training

Kenyan CDP staff attended a training course on bioinformatics tools for species identification with special focus on whitefly and CBSD genomic datasets during 7–10 June 2016 at JKUAT, Kenya. The workshop was facilitated by Dr Laura Boykin (UWA). The training focused on the use of software (e.g. Geneious) for sequence analysis and interpretation of phylogenetic trees. The trainees comprised Kenyan CDP members and postgraduate students as well as three scientists from CDP Zambia.

Recruitment of project staff

The project recruited seven individuals for different roles (Table 17). These comprised the country team leader, the assistant country team leader, one research assistant, a project technician and a driver. Two students (one PhD and one MSc) were also recruited and played key roles in project activities.

Table 17 Personnel recruited by CDP Kenya and respective roles

S/N	Name	Role
1	Elijah M. Ateka	Country Team Leader
2	Justus Onguso	Assistant Country Team Leader
3	Samuel Mwaura K.	Research Assistant
4	Timothy O. Makori	Project Technician
5	Benson Kobi Ongori	Project Driver
6	Martina Kyalo	PhD student
7	Brenda Muga	MSc student

Infrastructure strengthening

Laboratory and greenhouse construction

A Plant Virus Diagnostics Laboratory adjoined a greenhouse was constructed and completed in August 2016 and launched in October 2016 (Figure 23 and Figure 24). The laboratory is used primarily for CDP-related research and activities but is also available to other JKUAT staff and students for research and training (Figure 25). The laboratory is now being used by students and staff from JKUAT for research.

Figure 23 Chief guest unveils commemorative plaque (left) as guests tour the laboratory at JKUAT (right)

Figure 24 Group photo during the event (left) and the laboratory working space (right)

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Hope for Cassava Farmers as JKUAT Unveils Diagnostics Lab

Posted on October 5, 2016 by Corporate Communications Office

Dr. Mugiira (centre) unveils the plaque for the new laboratory as Prof. Odhiambo (second left) and Eng. Tanui (second right) witness

Efforts to realize sustainable cassava production and consumption in Kenya have been heightened following the launch of a plant virus diagnostics laboratory and greenhouse at Jomo Kenyatta University of Agriculture and Technology (JKUAT). The Ksh. 8 million facility which is supported by the Bill and Mellinda Gates Foundation (BMGF) through the Mikocheni Agricultural Research Institute, Dar es Salaam, Tanzania, is expected to foster national efforts in disease and insect vector mapping as well as analysis of emerging plant viruses.

The laboratory will also be used to produce high yielding, pest and disease free planting cassava materials besides teaching and research at the University.

It is estimated that over 90, 000 hectares are currently under cassava in Western, Nyanza and Coast regions with national annual production pegged at 540, 000 tonnes. The low yields averaging 10

News & Events

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Admitted KUCCPS Students – SEPTEMBER INTAKE 2018

Online Application and Admission Letters

Online Admissions Video Tutorial

Admission into Distant/Online

Figure 25 Article on the JKUAT website on the likely impact of the diagnostics laboratory at JKUAT

Equipment procured by the project in JKUAT Lab

Items procured for the laboratory at JKUAT during 2013–2017 (Table 18) were as follows:

Table 18 Assets purchased by CDP in Kenya and their description

S/No.	Asset description	Serial no.	Quantity	Date of purchase	Status
1	PCR machine	BYQ661501E-109	1	November 2016	Working
2	ELISA reader	Thermoscientific	1	February 2015	Working
3	Ultra Freezer-80	BDF86V158YSC16111510	1	January 2017	Working
4	Micro centrifuge	Not available	1	January 2017	Working
5	UV transilluminator	P/N 97-0274-08	1	November 2013	Working
6	Refrigerated centrifuge	Cat no 922215120000	1	April 2015	Working
7	Sterilizer	14347	1	November 2016	Working
8	Water bath	DSB-100	1	November 2013	Working
9	Ice maker	BL25A	1	November 2013	Working
10	pH meter	H12210	1	November 2013	Working
11	Water distiller	LWD30082016041903	1	November 2016	Working
12	Incubator/shaker	Not available	1	October 2016	Working
13	Microwave oven	Model ME731K	1	November 2013	Working
14	Laminar flow	ID No BS13V14120088D	1	May 2015	Working
15	Vortex Mixer	Model # M 37610-33	1	November 2013	Working
16	Weighing balance	Model TA302	1	October 2013	Working
17	Multichannel pipette	HB 10847	2	October 2013	Working
18	Gene gun	Not available	1	December 2015	Working
19	Toyota Hilux 4WD	KBU 799T	1	October 2013	Working

SECTION THREE: Impacts, success stories and learning outcomes

Impacts

Impact area	Impact
How many students were trained by this project directly and indirectly?	Four students were trained: two PhD and two MSc. One of the PhD students works for Biosciences eastern and central Africa Research Institute and one of the MSc students supports a project at JKUAT.
No of projects using the CDP facilities	Eight projects use the CDP facilities, including the bean, sweet potato, cassava, groundnut, eggplant, rice, cowpea and tamarind projects.
No of students and/or staff using facilities and reagents of CDP	Ten students use the facility currently. These students are from JKUAT, Pan African University and Makerere University.
No. of people that have been inspired by the project	Over 100 people have been inspired: from KALRO, Pan African University, University of Nairobi, Kenya Plant Health Inspectorate Service (KEPHIS) and University of Eldoret. Some of these scientists have expressed willingness to collaborate, visit our laboratory facilities or called to request clean planting materials.
Institutional visibility	The laboratory and greenhouse facilities are being used by researchers from other institutions and projects, e.g. Makerere University, Pan African University and KEPHIS.
Infrastructural capacity – helping student execute their project	Assisted nine students with their work on different crops including beans, cassava, groundnut, eggplant, rice, cowpea and tamarind.
New stakeholders interacting with the project	Pan African University (5) and KEPHIS (2).
Service the laboratory has provided	Training for postgraduate students takes place every semester. Training of 20 visiting scientists from University of Eldoret in nanopore sequencing (March 2018).
How many farmers have benefited either directly or indirectly	Over 4300 farmers benefited by getting direct advice and clean planting materials.
New collaborations/collaborative projects	CDP Kenya collaborated with four research projects.
People using information generated by this project	Breeders, plant pathologists and students used disease and virus strain distribution maps for cultivar deployment.
Benefits to the government -extension training, inspectors and regulators	There were 84 extension workers trained. We provided information to more than 100 extension officers and 28 policy makers during shows and exhibitions.
Advocacy impacts on policy	The CDP team contributed to the formulation of the national cassava development strategy and proposed the

	necessity of testing cassava planting materials marketed as seed.
Publications and other communications including other communication materials	Eight published peer-reviewed articles 1500 brochures issued Three manuscripts in preparation Two magazine articles One newspaper article.
Increase in crop yield and incomes (especially farmers who used clean materials)	Up to a four-fold yield increase as a result of using clean material as seen in the demonstration trials.
Meetings and conferences attended – for the whole team	The team attended all annual CDP project meetings, two agricultural shows each year, and the annual JKUAT conference every year.
Support to breeders and other projects	We supported two cassava breeders in symptom recognition and particularly scoring for incidence and severity. CDP supported indexing of seven varieties.
Involvement of vulnerable groups	We involved six farmer groups in the demo plot trials which comprised small-scale farmers, predominantly women. A group comprised 30–50 individuals.
Change of farmers perceptions	Farmers are more aware of virus diseases and are more willing to buy clean/tested planting materials.
Other universities requesting to use the facilities/equipment	The Pan African University, Nairobi.
Minimizing/arresting brain drain	For 5 years no scientist involved in CDP left the institution (JKUAT) either for economic or intellectual reasons.
Building a network of scientists	A strong network established among CDP scientists (in CDP partner countries) and with other Kenyan scientists from KALRO, KEPHIS, Ministry of Agriculture, Nairobi University and Eldoret University. These include the CDP partners.

Success stories

JKUAT received a boost through the establishment of an equipped diagnostics lab with an adjoined greenhouse which not only facilitated cassava and project work but is also being used for the teaching and training of other students working on other projects and crops.

Four postgraduate students were trained with direct support of this project. This supported the country's capacity to diagnose and manage plant virus diseases. The team can now diagnose virus diseases using molecular based tools. Training in practical laboratory skills increased the competencies and confidence of Kenyan scientists to conduct research previously considered a preserve of advanced labs.

The project reached over 4300 farmers through various trainings and interactions. Many farmers can now identify CMD and CBSD and are willing to pay for clean planting material. Similarly, a total of 84 extension staff were trained in CMD and CBSD symptom identification and management. The extension officers agreed to sensitize farmers and seed merchants to use virus-tested material whose

origin was known. This has resulted in the decline in CMD and CBSD incidence across the country over the project period (2013–2017).

Information was widely shared including eight publications in peer refereed journals within the duration of the project. This increased the project's visibility.

Additionally, several collaborations have been forged with scientists locally and internationally. For instance, there has been collaboration with scientists from Australia (UWA), Tanzania, University of Cambridge, Makerere University, Uganda and Rothamsted Research, UK.

Learning outcomes

Change in farming practices

From the outreach activities, farmers are now more aware of the causes and management strategies for managing virus diseases in cassava. Accordingly, their practices have changed as evidenced by their increased demand for clean planting materials and willingness to buy clean seed.

Increased effectiveness of extension officers

There is increased knowledge and awareness among extension workers on virus diseases. They are now better placed in the long term to assist farmers within their geographical areas.

Plant breeding and disease management

Knowledge on the occurrence and distribution of viruses and virus strains has informed breeders and pathologists on what varieties to breed for and the strategies for germplasm deployment.

Strengthened linkages

We learned the importance of researcher–extension–farmer linkages to manage the spread of cassava diseases. Through our work with extension officers and farmers we now have a pool of knowledge that will benefit cassava production.

Data collection

Initially, field survey data recording was paper-based. We learned that this method is error-prone and we worked with our collaborators in the UK to develop an electronic application.

Laboratory management

We learned to plan our procurement requirements more carefully to take account of the delays that can occur between the ordering of equipment and reagents, and their arrival at the laboratory.

Conclusion

Our investigations showed that CMBs are no longer geographically localized and are found in all cassava-growing areas. The geographical overlap of these viruses in this survey is most likely associated with the unchecked movement of infected planting materials. These findings highlight the importance of screening and controlling the movement of planting materials. The CBSV and UCBSV did not have any specific distribution pattern, although both were absent from the Eastern region. Eight new genomes from Kenyan cassava samples were characterized; this was achieved through improved molecular procedures. This important step creates opportunities for the development of better diagnostic tools, particularly primers, for CBSV detection.

Another method for the detection of CBSV, LAMP, was validated. The procedure was more specific and more sensitive than RT-PCR and could therefore be recommended for routine detection of CBSV and UCBSV. However, more work needs to be done to further adapt the test to field conditions.

Our investigation to determine alternative host plants identified the infection of *Markhamia zanzibarica* by EACMV-Ug for the first time. This is vital information that will assist the sustainable management of CMD as it could lead to a better understanding of disease epidemiology.

The capacity of the country to diagnose and characterize disease pathogens was greatly improved through the establishment of a diagnostics lab and greenhouse. This facility is now being used by students and scientists working on other projects and crops. This increase in competence was also due to the training of four postgraduate students and Kenyan scientists.

The training for farmers and agricultural extension staff on the identification of CMD will be a boost to efforts to manage these diseases. This could result in the decline of CMD and CBSD incidence across the country.

The CDP met its objectives and has become an example to other projects to emulate within Kenya and East Africa. The project adopted a holistic approach as we not only tried to understand the threat from the evolving viruses and vectors and build sustainable regional capacity, but we catered for the needs of small-scale farmers by providing a clean seed system for those who were almost giving up on cassava production in Kenya.

Publication of research findings

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Interaction of *East African Cassava Mosaic Virus*-Uganda and Cassava brown streak viruses in cassava varieties with varying levels of resistance. In preparation.

Acknowledgements

This work was supported by the Tanzania Agricultural Research Institute (TARI)–Mikocheni with joint funding from the Bill & Melinda Gates Foundation (BMGF) and UK Department for International Development (DFID) through the 'Cassava disease diagnostics for sustainable productivity in Africa' project (Grant no. 51466).

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RWANDA

Marie Claire Kanyange[1], Gervais Gashaka[1], Esperance Munganyinka[1], Marie Mutumwinka[1], Ghislain Niyonteze[1], Jeanine Umfuyisoni[1], Peter Sseruwagi[2], Fred Tairo[2] and Joseph Ndunguru[2]

[1] Rwanda Agriculture Board, P.O. Box 5016, Kigali, Rwanda
[2] Tanzania Agriculture Research Institute (TARI)–Mikocheni, P.O. Box 6226 Dar es Salaam, Tanzania

Abstract

The Cassava Diagnostics Project (CDP) aimed to (a) understand the threat from evolving viruses and vectors, (b) reach farmers directly and through partners, and (c) build sustainable regional capacity. The occurrence and distribution of cassava virus diseases – cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) – and the causal viruses in Rwanda were determined through three surveys in 2013, 2015 and 2017. The surveys were conducted in 10 major cassava-producing districts including: Bugesera, Kayonza, Kirehe and Nyagatare (Eastern Province), Kamonyi, Ruhango, Nyanza and Gisagara (Southern Province), Rusizi and Nyamasheke (Western Province) Districts. A total of 410 smallholder cassava fields were assessed for CMD and CBSD incidence and symptom severity, and adult whitefly abundance.

Mean CMD incidence ranged within 6.7–81.7% across the provinces sampled, and mean CBSD incidence within 0–20.5%. Molecular analysis results of the cassava mosaic begomoviruses (CMBs) affecting cassava in Rwanda indicated that *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV) occurred at very low levels – 0% and 1.18% of the samples, respectively. No co-infections of ACMV + EACMV were detected. The RT-PCR analysis showed the presence of two viruses causing CBSD: *Cassava brown streak virus* (CBSV) and *Uganda cassava brown streak virus* (UCBSV).

Farmers, extension staff and stakeholders were trained in 'training of trainers' workshops. Demonstration plots were established to show the benefits of using disease-free planting materials in pest and disease management. There were 1400 cassava stems distributed to smallholder farmers. With respect to raising awareness, leaflets were developed in the local language (Kinyarwanda) to help farmers gain a better understanding of the messages and information on cassava viral diseases, the whitefly vector and the management strategies.

Capacity was strengthened considerably in Rwanda through the training of one PhD and three MSc students. In addition, the Country Team Leader, her assistant and a Research Assistant received short-term training in a number of areas to enhance their performance of project activities. Training was provided for stakeholders including extension staff, agricultural officers, private company staff and smallholder farmers on field diagnosis and management of cassava viral diseases. Infrastructural capacity was enhanced though the acquisition of laboratory equipment and reagents to conduct molecular diagnostics.

Acronyms and abbreviations

ACMV	*African cassava mosaic virus*
CBSD	Cassava brown streak disease
CBSV	*Cassava brown streak virus*
CDP	Cassava Diagnostics Project
CMB	Cassava mosaic begomoviruses
CMD	Cassava mosaic disease
CTL	Country Team Leader
EACMV	*East African cassava mosaic virus*
ISAR	Institut des Sciences Agronomiques, du Rwanda
MAP	Months after planting
RAB	Rwanda Agricultural Board
SOP	Standard Operating Procedure
TARI	Tanzania Agriculture Research Institute
UCBSV	*Uganda cassava brown streak virus*

Results summary: Rwanda

Aim I: Understand the threat from evolving viruses and vectors

Objective 1: Disease epidemiology

Disease and whitefly prevalence surveys conducted	• Four surveys conducted in 2009, 2013, 2015 and 2017, covering 410 fields in 14 districts. • Survey data for 2009, 2013 and 2015 were cleaned and validated by Tanzania Agriculture Research Institute (TARI)–Mikocheni and Cambridge modeling teams during the data entry and assembly workshop held from 27 November to 3 December 2016 in Dar es Salaam, Tanzania. • A new SOP for 2017 countywide survey developed and used to collect 2017 data. • Cassava leaf samples with Cassava mosaic disease (CMD) and Cassava brown streak disease (CBSD)-like symptoms, were collected for laboratory analysis.
Relationship between CBSVs presence/load and disease symptom development determined	• One manuscript published by Esperance Munganyinka from her PhD research (Munganyinka et al., 2018). • Participated in the AgShare.Today Scientific Writing Workshop in Lusaka, 2–7 October 2017. • Currently engaged on Objective 2 to quantify the viral loads of *Cassava brown streak virus* (CBSV)-infected plants in the DSMZ laboratory under the supervision of Dr Stephan Winter in Germany.
Spread of CBSD within and between cassava fields	• Ms Jeanine Umfuyisoni defended her MSc dissertation successfully in 2018 at Makerere University.
Alternative hosts for CBSVs and CMBs and associated insect vectors identified	• CBSV whole genome sequences obtained from non-cassava shrubs in Rwanda as part of Ms Jeanine Umfuyisoni's MSc research. • One manuscript by Ms Umfuyisoni is in preparation to report on her MSc research.

Objective 2: Characterization of emerging viruses

Cassava virus isolates in the project countries sequenced and analyzed	• CBSV and *Uganda cassava brown streak virus* (UCBSV) whole genomes were obtained and their genetic diversity studied.
Cassava virus distribution maps for partner countries, generated (incidence, severity, whitefly)	• Disease distribution maps produced and distributed to stakeholders.

Objective 3: Characterization of disease vectors

TASKS	RESULTS
Whiteflies characterized	• Whitefly samples were collected and characterized – identifying the Rwanda species as SSA1.

Aim II: Support clean seed systems for farmers	
Objective 6: Conventional breeding support	
Breeders' material monitored for disease and indexed for virus (CMBs and CBSVs) load at 3, 6, 9 and 12 months after planting (MAP)	• Twelve genotypes were indexed for cassava mosaic begomoviruses (CMBs) and CBSVs. These were promoted for disease management.
Objective 9: Reaching farmers directly and through partners	
Farmers trained on CMD and CBSD disease symptom recognition and management strategies	• Thirty farmers trained in CMD and CBSD symptom recognition and management at workshops.
Demonstration plots for benefits of using virus-indexed planting materials established on-farm	• Two demonstration plots were set up to train farmers in best practices to manage cassava diseases. Clean planting materials (1400 stems) distributed to farmers.
Information materials developed and disseminated	• There were 150 leaflets relating to cassava diseases and management printed and distributed to farmers and extension officers. • Two radio programs on cassava diseases and their management were aired.
Aim III: Build sustainable regional capacity	
Objective 10: Strengthening stakeholder linkages	
Team leader meeting to develop country-specific milestones	• Rwanda Country Team Leader (CTL) and Assistant CTL participated in the development of country-specific milestones.
Project inception and consultative meeting with stakeholders conducted	• Rwanda CTL and Assistant CTL participated in project inception and consultative meetings.
Exchange visits between scientists in the project countries conducted	• Ms Marie C. Kanyange (CTL) and Assistant CTL, Mr Gervais Gashaka, participated in the first exchange visit to Zambia Agriculture Research Institute during 15–21 May 2016. • The team visited the Cassava Diagnostics Project (CDP) research activities in Zambia including the new screenhouse and laboratories, and farmers' fields to assess the disease situation.
Awareness on availability of diagnostic capacities created through training and different media	• Awareness in diagnostic capacity was provided by CDP researchers to Rwanda Agricultural Board (RAB) scientists, university students and private-sector companies.

Objective 11: Strengthening human capacity and infrastructure	
Human capacity	
Project staff recruited	• Seven staff: one driver, one PhD and two MSc students, one Research Assistant, one Assistant CTL and one CTL.
PhD and MSc trained on different aspects of cassava virus diseases	• One PhD and one MSc student trained.
Advanced specialized training and visits for project scientists (1–2 months) conducted	• One staff member, Ms Marie C. Kanyange (CTL) together with other CTLs and TARI–Mikocheni project management team visited the Agricultural Research Organisation of Israel during 2–8 February 2014. • CTL visited Rutgers University, USA, to learn analysis of begomoviruses in Rwanda in June 2015 with the instruction of Prof Siobain Duffy.
Project staff trained on IP, biosafety issues and communication strategies	• CTL attended training in intellectual property and communication strategies during 27–31 October 2014 at the World Agroforestry Center (ICRAF), Nairobi, Kenya.
Project results and information disseminated	• One manuscript published in 2018 by PhD student Esperance Munganyinka – on relationship between CBSV presence and disease development. • One manuscript published in 2018 by MSc student Concilie Nyirahorana on influence of CBSD control measures on cassava production in Bugesera and Ruhango Districts • Four annual meeting reports.
Institute Directors trained in Leadership and management	• One Director of RAB attended the 'Leadership Skills for Institutional Directors' conducted in July 2014 in Entebbe, Uganda, and Kigali, Rwanda.
Infrastructure strengthening	
Greenhouses constructed/renovated	• One screenhouse renovated at Rubona and is in use.
Vehicles, laboratory equipment and consumables procured	• One project vehicle procured and in use. • Various equipment procured and in use.
Project management	• One RAB Institute Accountant, participated in the workshop on accounting package TALLY, during 14–17 May 2013 in Dar es Salaam, Tanzania. • The CTL, Ms Marie C. Kanyange, participated in the financial management training workshop in Kigali, Rwanda, in 2015. • CTL and two students attended the AgShare.Today training and scientific report writing skills in January 2016 in San Diego, USA.

Background

Cassava was introduced to Rwanda around 1932 and is grown across the country in North Province, East Province and West Province (Figure 1). It is an important staple food and is currently being promoted as a cash crop through the establishment of cassava processing plants. In addition to its tuberous root, its leaves are treated as a vegetable called isombe, which is rich in protein and vitamins. Cassava is consumed in various forms (raw, paste/bread or ugali, boiled for breakfast, mixed with beans, vegetables, etc.) and its cooking and preparation methods vary from one individual to another. Although cassava is a major food crop, its production is threatened by the two most devastating viral diseases: cassava brown streak disease (CBSD) and cassava mosaic disease (CMD). Since 2015, cassava yield has declined considerably, due to the pandemic of CBSD and CMD, a collapsed seed system and supply mechanism, low productivity, poor market linkage between producers and processors, and poor agricultural practices and processes.

The CBSD poses a serious threat to cassava productivity in East and Central Africa – it was first reported in 1936 in Tanzania and currently occurs in almost all East and Central African countries. In Rwanda, the disease was first reported by the Institut des Sciences Agronomiques du Rwanda (ISAR) in 2009 (ISAR, 2009 unpublished data) and has since been reported by the Rwanda Agricultural Board (RAB) to have spread widely in the main cassava growing areas of the country: Southern and Eastern Provinces (RAB, 2012 unpublished data).

Little work has been done in Rwanda to document the distribution, incidence and severity of CBSD; hence there is limited knowledge on the viruses responsible for the disease and their genetic diversity and distribution in the country. In East Africa, some epidemiological studies on CBSD have been undertaken, but significant knowledge gaps exist due to the variability in patterns of disease symptom expression. In addition, it is not clear whether symptom development is dependent on the virus species – *Uganda cassava brown streak virus* (UCBSV) or *Cassava brown streak virus* (CBSV) – or virus load or virus localization. There is a need to raise the level of knowledge on the disease, since this is crucial for developing effective and efficient diagnostic and management tools – this was achieved in one PhD student's research.

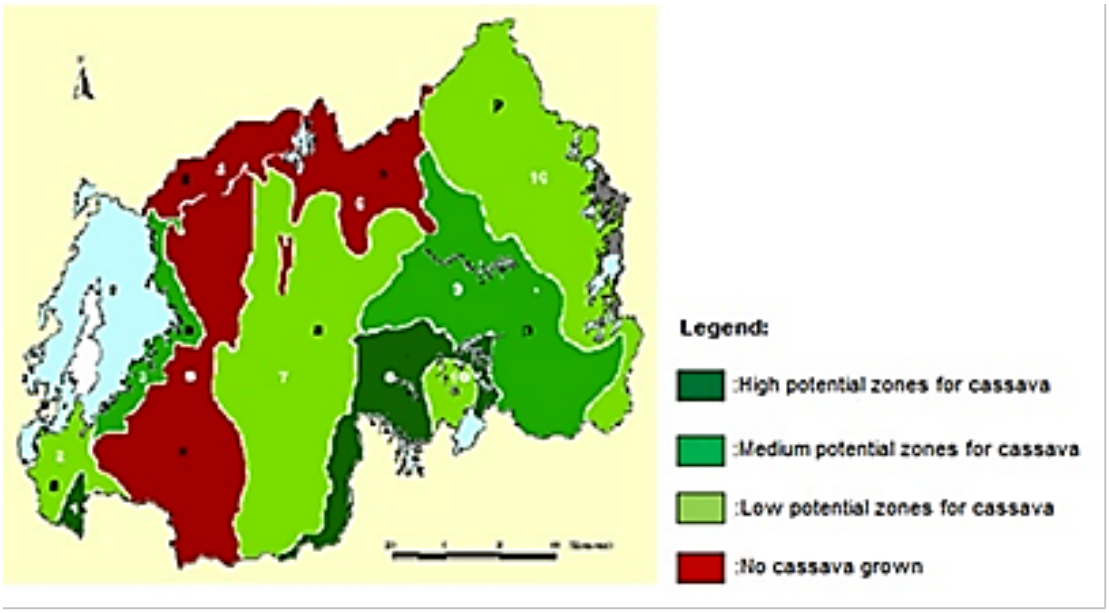

Figure 1 Major agro-ecological zones in Rwanda (adapted from Bazarusanga et al., 2011)

Under ideal growing conditions, disease-free cassava can yield more than 30 t/ha. However, despite attributes such as drought tolerance and low input requirements, yield is poor in Rwanda with 8–10 t/ha.

SECTION ONE: Understanding the threat from evolving viruses and vectors

Disease epidemiology

Four countrywide disease monitoring surveys were carried out in 2009, 2013, 2015 and 2017. In 2009, fields were assessed 3–6 months after planting (MAP) for CMD and after 6 MAP for CBSD. The 2013 survey was conducted during July–August in seven cassava growing districts: Kayonza, Kirehe, Nyagatare and Bugesera (Eastern Province); and Ruhango, Nyanza and Gisagara (Southern Province). A total of 67 and 137 fields were surveyed for CMD and CBSD, respectively, and 612 samples were collected for virus analysis. In 2015, a countrywide survey was conducted during May–June, with a total of 100 and 193 fields surveyed for incidence and severity of CMD and CBSD, respectively. About 879 samples were collected for virus analysis from 10 districts: Kayonza, Kirehe, Nyagatare and Bugesera (Eastern); Kamonyi, Ruhango, Nyanza and Gisagara (Southern); and Rusizi and Nyamasheke (Western). Another countrywide survey was conducted in June 2017. A total of 100 and 137 fields were surveyed for incidence and severity of CMD and CBSD, respectively. A total of 705 samples were collected for virus analysis from Kayonza, Kirehe, Nyagatare and Bugesera (Eastern); Kamonyi, Ruhango, Nyanza and Gisagara (Southern); and Rusizi and Nyamasheke (Western). The whitefly population was assessed by counting numbers of adult whitefly on the five upper fully expanded apical leaves and samples collected in 80% ethanol for species identification and genetic diversity analysis. Geo-coordinate points for each field were taken using a GPS receiver to produce distribution maps for cassava diseases and whitefly abundance in Rwanda.

CMD incidence and symptom severity

In 2009, CMD incidence in Eastern districts ranged within 30.8–42.2%; in Southern districts it was 11.7–81.7% and in Western districts was 6.7–44.0% (Figure 2). Incidence and severity distribution maps from this survey are shown in Figure 3.

In 2013, CMD incidence was moderate (20.7%) in Nyagatare District, but low in the remaining districts (<10%). The moderate incidence in Nyagatare was probably due to the predominant use of local varieties susceptible to CMD. The CMD severity was severe (>3) in all districts except Gisagara (2.8). Cassava fields with high CMD incidence were more prevalent in the south and north than in the east. The distribution of cassava fields with severe CMD followed a similar trend.

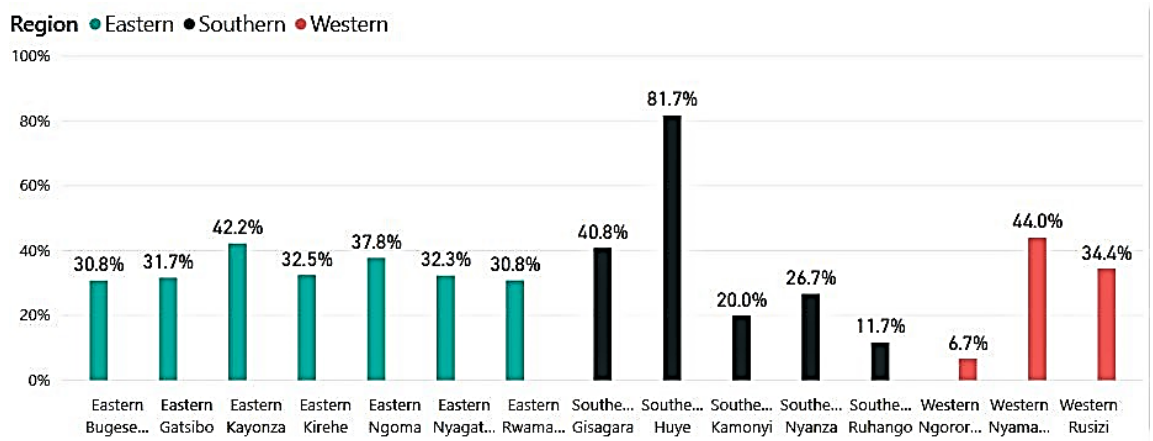

Figure 2 CMD incidence in districts – 2009 survey

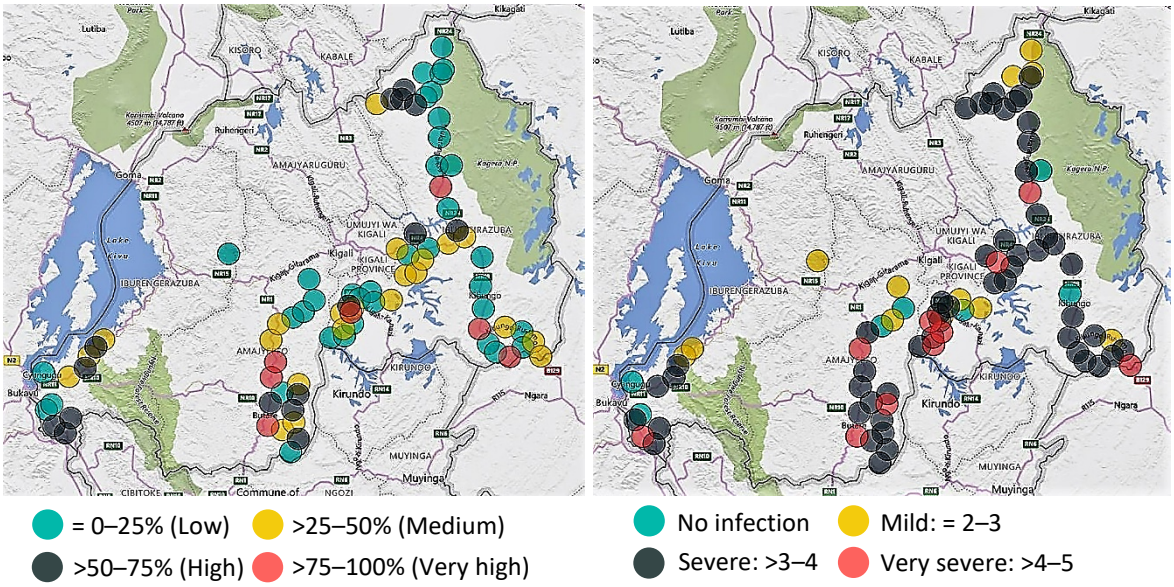

Figure 3 CMD – 2009 survey: (left) incidence and (right) severity

In 2015, CMD incidence in Eastern districts ranged within 0–33.8%, in Southern districts it was 0–9.6% and in Western districts was 4.2–10% (Figure 4). Incidence and severity distribution maps from this survey are shown in Figure 5. CMD incidence was high (>50%) in Nyagatare District, and moderate (20–40%) in Kayonza and Kirehe Districts but was low in the remaining districts (<20%). The CMD severity was severe (>3) in all Eastern districts, and in Nyamasheke District of Western Province. Most cassava fields had low to moderate disease incidence and mild symptoms in the surveyed areas.

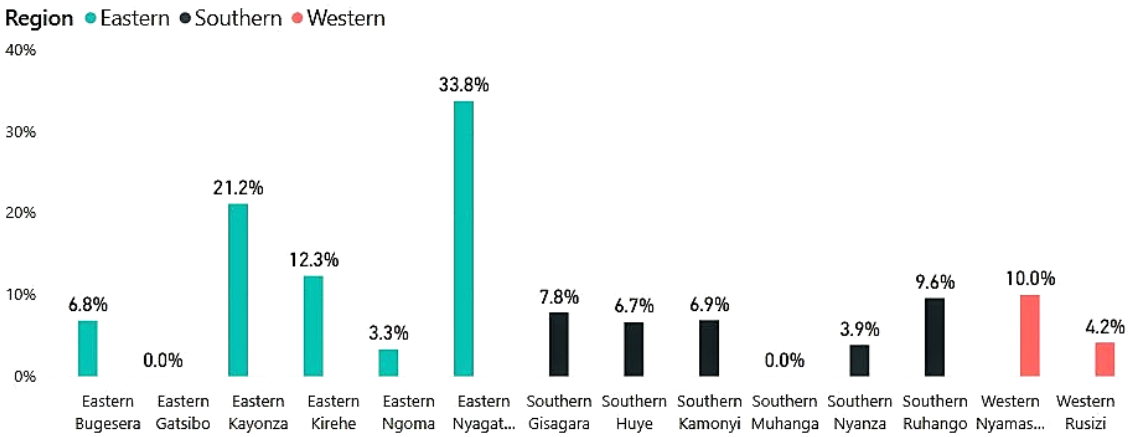

Figure 4 CMD incidence in districts – 2015 survey

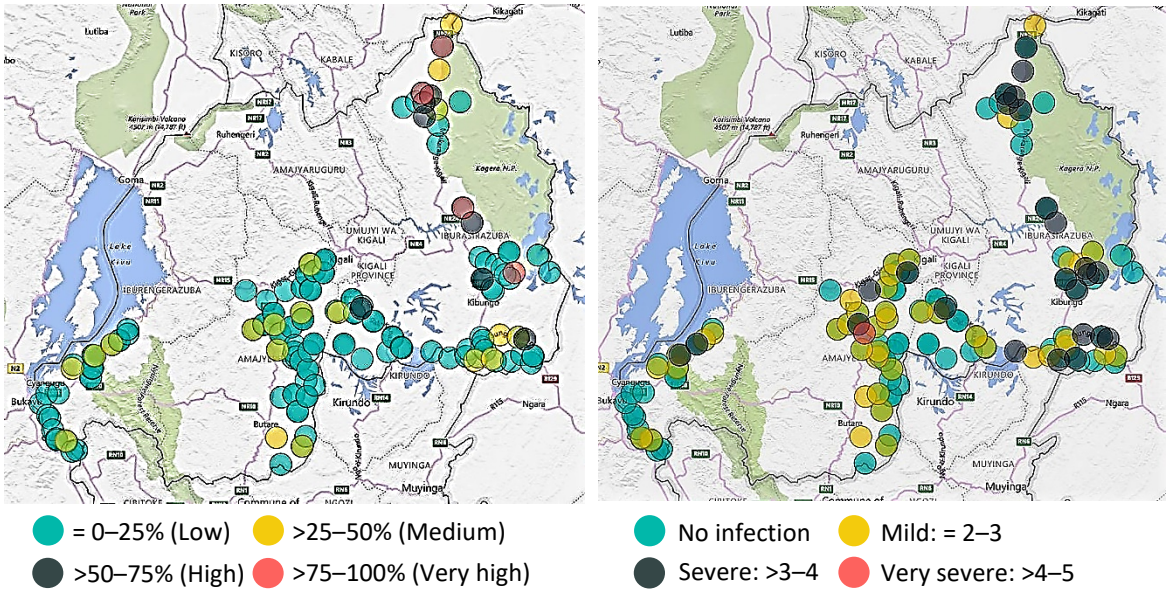

Figure 5 CMD – 2015 survey: (left) incidence and (right) severity

CBSD incidence and severity

In 2009, CBSD incidence in Eastern districts ranged within 0–20.5%, in Southern districts was 0–10% and in Western districts was 0–1.1% (Figure 6). Incidence and severity distribution maps from this survey are shown in Figure 7.

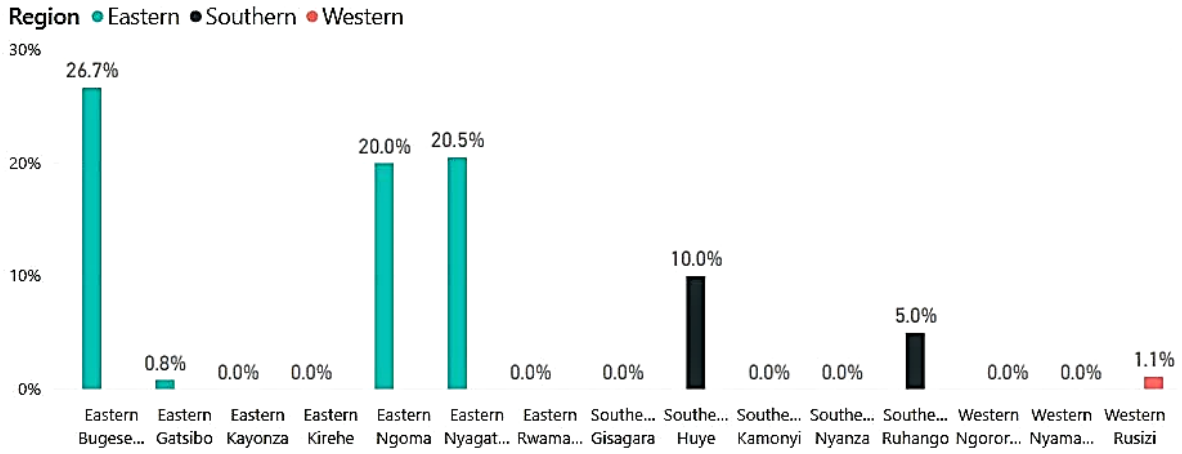

Figure 6 CBSD incidence by district – 2009 survey

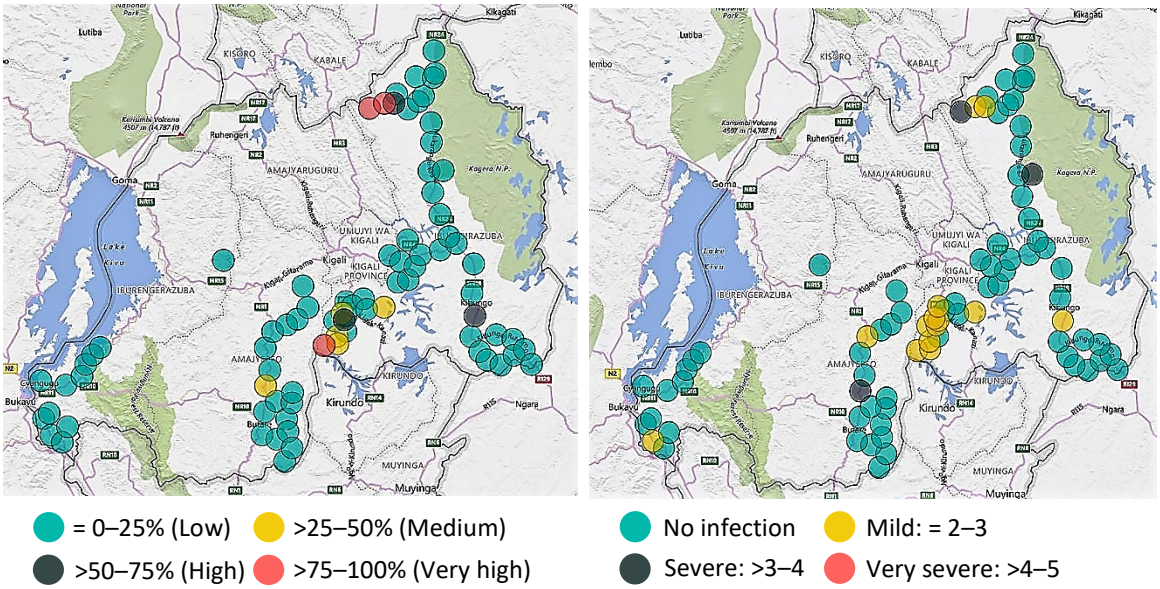

Figure 7 CBSD – 2009 survey: (left) incidence and (right) severity

In 2013, mean CBSD foliar incidence was very low (4–12%) in all districts; however, mean root incidence was high (52–66.6%) in Bugesera, Kirehe, Nyagatare and Ruhango Districts. The CBSD symptom severity was assessed on foliage, stems and roots. There were no symptoms on stems in all districts. The CBSD severity on foliage was in the range of 2.0–2.5 in Bugesera, Kirehe, Nyagatare, Ruhango and Nyanza. The severity on roots was moderate and ranged within 2.1–2.5 in all districts.

In 2015, CBSD incidence in Eastern districts ranged within 0–37.7%, in Southern districts was 20–77.8% and in Western districts was 4.2–10.8% (Figure 8). Incidence and severity distribution maps from this survey are shown in Figure 9. Foliar incidence of CBSD was high (>40%) in Kamonyi, Ruhango, Nyanza Gisagara and Bugesera. The CBSD root necrotic symptoms were high (>30%) in Bugesera, Kamonyi, Nyanza, Ruhango and Gisagara Districts. The CBSD leaf symptoms were severe (3–4) in Nyamasheke and Gisagara. The CBSD root necrosis was severe (>3) in Kirehe, Kamonyi, Ruhango and Nyanza.

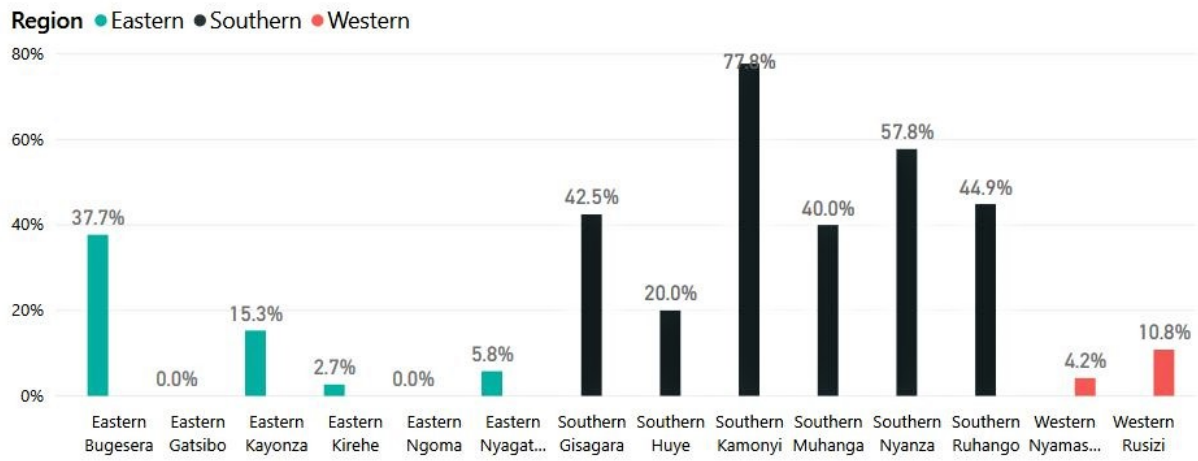

Figure 8 CBSD incidence by district – 2015 survey

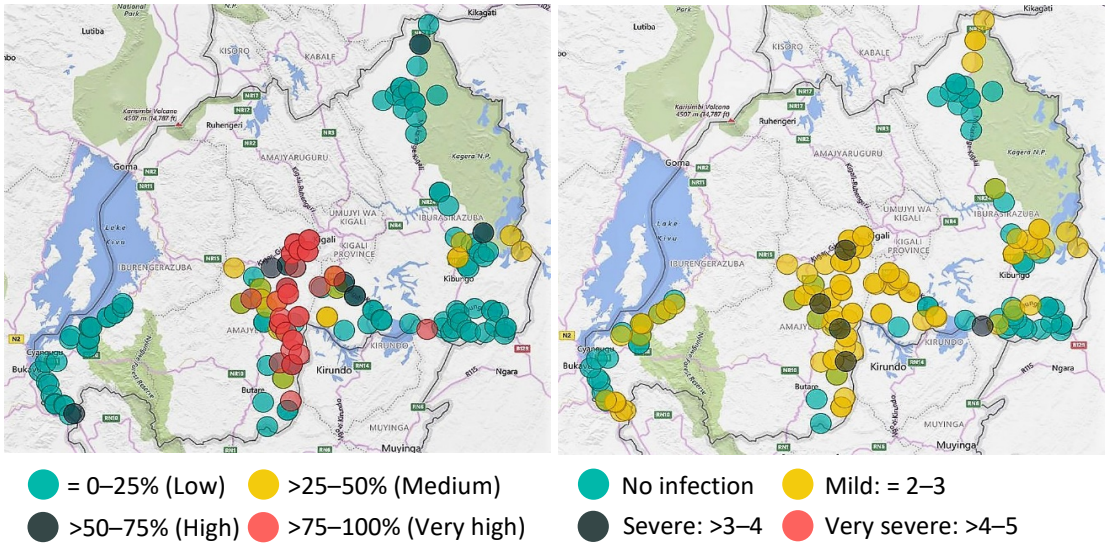

Figure 9 CBSD – 2015 survey: (left) incidence and (right) severity

In 2009, mean whitefly abundance was 2.77 (52 fields), 0.46 (18 fields) and 1.04 (12 fields) in Eastern, Southern and Western provinces, respectively. In 2013, the mean whitefly abundance was 31.64 (62 fields) and 61.24 (41 fields) in Eastern and Southern Provinces, respectively. In 2015, mean whitefly abundance was 29.63 (73 fields), 23.07 (61 fields) and 0.52 (28 fields) in Eastern, Southern and Western Provinces, respectively.

Characterization of emerging viruses

Cassava virus isolates sequenced and analyzed

Plant material from the countrywide field surveys were subjected to molecular tests using primers defined in Mbanzibwa et al. (2009). Molecular characterization of CBSVs was done for 300 samples. Analyses revealed the presence of CBSV and *Uganda cassava brown streak virus* (UCBSV). The RT-PCR analysis for CBSVs showed high levels of CBSV infection (10%) followed by UCBSV (9.67%) and 1.67% co-infected plants. Co-infection was found in Southern Province (Nyanza and Gisagara Districts) and Eastern Province (Bugesera District) (Table 1).

Table 1 Detection of CBSVs in Rwanda, 2015

District	Number of samples	CBSVs			Total
		CBSV	UCBSV	CBSV + UCBSV	
Kayonza	30	3	0	0	**3**
Kirehe	30	0	0	0	**0**
Nyagatare	30	0	0	0	**0**
Bugesera	30	2	4	1	**7**
Kamonyi	30	9	1	0	**10**
Ruhango	30	6	2	0	**8**
Nyanza	30	7	9	3	**19**
Gisagara	30	3	8	1	**12**
Nyamasheke	30	0	0	0	**0**
Rusizi	30	0	5	0	**5**
Total	**300**	**30**	**29**	**5**	**64**
Percent		**10.0**	**9.7**	**1.7**	**22.9**

Two cassava mosaic begomovirus (CMB) species: *African cassava mosaic virus* (ACMV) and EACMV were detected in the samples tested in 2013 and 2015. No co-infections were found in 2013; however, in 2015, co-infections were detected (approximately 1%; Table 2). The 2013 survey showed the presence of EACMV in all districts except Ruhango and Kayonza. In 2015, the survey indicated both EACMV and ACMV in different districts, with EACMV present in all districts except Nyanza, Rusizi and Nyamasheke, and ACMV present in Nyanza and Nyamasheke.

Table 2 Summary of CMB detections in 2013, 2015 and 2017 in Rwanda

Year	ACMV	EACMV	ACMV+ EACMV	Total
2013	0	18 (1.18%)	0	152
2015	6 (2.15%)	25 (8.96%)	3 (1.07%)	279

Epidemiology and distribution of CBSD in Rwanda

In Rwanda, recent research efforts focused on CBSV detection and determination of incidence, severity and whitefly counts by conducting diagnostic surveys. However, the Rwandan isolates of CBSVs were unknown, representing a big gap in knowledge of epidemiology and management of CBSD in Rwanda. Also, there have been no reports on the potential roles of non-cassava plant species acting as virus reservoirs or alternative hosts in the perpetuation of CBSVs in Rwanda and at region level. Increased availability of sequences of isolates from wild species may provide more information on the evolution of CBSV and UCBSV and thus improve our awareness of the adaptation of these viruses to cassava as a new host. It is therefore very important to know the different isolates of CBSD causal agents in cassava and identify the alternative hosts for CBSV and UCBSV, and then develop appropriate diagnostic tools for proper management of CBSD in Rwanda. This work was addressed by one of our MSc students, Mrs Jeanine Umfuyisoni.

Based on 2015 cassava disease survey data from young cassava fields (3–6 MAP), the study established that CBSD incidence and severity had increased from 18.8% and 2.1 in 2012, respectively, to 27.39% and 2.4 in 2015. The disease occurred at a moderate severity score that had increased by 0.3 (using a scale of 1–5) during the three years. The study also established the diversity of CBSVs affecting cassava crops in Rwanda and at least five different isolates were confirmed to be the main CBSD causal agents and indicated a greater genetic variability among UCBSV than CBSV isolates. This fact, coupled with the much wider distribution of UCBSV than CBSV, suggests that UCBSV is likely to be the endemic species associated with cassava in Rwanda. Five non-cassava plant species were identified as CBSV hosts in Rwanda. This is an important factor in designing proper management strategies for cassava viral diseases.

The relationship between CBSV presence and disease development

The main objective of the study contributes to the knowledge of CBSV biology and epidemiology. This work was part of the PhD research of Ms Espérance Munganyinka (Jomo Kenyatta University of Agriculture and Technology, Kenya), and the findings are published in Munganyinka et al. (2017).

Characterization of disease vectors

Whitefly samples were collected and characterized using the *mtCO1* gene. The whitefly species was identified as SSA1.

SECTION TWO: Integrated pest management

Conventional breeding support

Breeders' material monitored for disease

After harvesting the advanced yield trial crop (AYT), 11 clones were tested for uniformity yield trials (UYT) in different locations in 2016. The results at 6 MAP showed that CMD and CBSD incidence and severity had different levels on improved varieties. The incidence and severity varied among locations: high at Karama and Rubona but relatively low at Nyagatare. At Karama Research Station, the highest CBSD incidence was 36.4% on leaves and the highest severity score was 3 on leaves. The highest incidence of CMD was 28.4% and the highest severity score was 2.3 (Table 3). At Nyagatare Research Station, the clone with the highest CBSD incidence had 25.8% on leaves and mean severity of 2; the highest incidence of CMD was 3.1% and the highest severity score was 2 (Table 4). At Rubona Research Station, the highest incidence was 8.1% with severity score of 2.5 for CMD and the high incidence was 26.6% with severity score of 2 for CBSD (Table 5).

Table 3 Incidence and severity of CMD and CBSD at Karama Research Station at 6 MAP (UYT)

No.	Names of clones	CMD incidence (%)	CMD severity	CBSD incidence (%)	CBSD severity
1	01/1371-5	0	1	2.9	2
2	TME 419/60	1	2	0	1
3	05/0099/17	0	1	13.8	2.7
4	UNKNOWN 1	8.3	2.3	36.4	3
5	UNKNOWN 2	0	1	11.5	2.5
6	06/2357-2	0.8	2	1.9	2.5
7	05/0127/35	8.1	2	8.2	2
8	01/1206/75	0	1	0	1
9	06/1630-1	0	1	4.3	2
10	GARUKUNSUBIRE	2.9	2	21.8	2.3
11	05/1814-4	28.4	2.5	15.8	2
12	01/1412/63	4.4	2	2.9	2

Table 4 Incidence and severity of CMD and CBSD at Nyagatare Research Station at 6 MAP (UYT)

No.	Names of clones	CMD incidence (%)	CMD severity	CBSD incidence (%)	CBSD severity
1	01/1371-5	0	1	0	1
2	TME 419/60	0	1	0	1
3	05/0099/17	0	1	0	1
4	UNKNOWN 1	0	1	0	1
5	UNKNOWN 2	0	1	4.5	2
6	06/2357-2	0	1	4	2
7	05/0127/35	0	1	0	1
8	01/1206/75	0	1	25.8	2
9	06/1630-1	0	1	0	1

No.	Names of clones	CMD incidence (%)	CMD severity	CBSD incidence (%)	CBSD severity
10	GARUKUNSUBIRE	3.1	2	0	1
11	05/1814-4	0	1	3.8	2
12	01/1412/63	0	1	0	1

Table 5 Incidence and severity of CMD and CBSD at Rubona Research Station at 6 MAP (UYT)

No.	Names of clones	CMD incidence (%)	CMD severity	CBSD incidence (%)	CBSD severity
1	01/1412/63	0	1	9	2.3
2	TME 419/60	0	1	11.3	3.3
3	Unknown 1	2.4	2.1	11	3
4	Unknown 2	0	1	4.6	2.1
5	05/0127/35	0	1	13.7	2.6
6	01/1371-5	0	1	2	4
7	06/1630-1	0	1	19	4.6
8	05/0099/17	0	1	26.6	2.6
9	CYIZERE	8.1	2.5	11	2.8
10	01/1206/75	0	1	6.5	2
11	06/2357-2	3	2	7.4	2.5
12	05/1814-4	0	1	9.8	2.8

After harvesting CET, 11 clones were advanced at PYT for further testing in different locations in 2017. At 6 MAP, CMD and CBSD incidence and severity showed different levels on improved varieties. The incidence and severity varied between locations: high at Karama and relatively low at Rubona. The following clones were evaluated: MM06/0090-OP/12, MM06/0128-OP/1, MM06/0128-OP/4, MM06/0128-OP/3, MM06/0128-OP/10, Bulk/35, Bulk/16, NASE14 (Check), MH05/0091-OP/11, Bulk/13, MH05/0091-OP/12 and NAROCASS1. At Karama, two clones (MM06/0128/1 and Bulk/35) out of 11 showed no CMD or CBSD leaf symptoms at 6 MAP. Three clones showed CBSD symptoms on leaves. The clone with highest CBSD incidence had 50% on leaves and the clone with the highest CBSD severity had a score of 3. The highest incidence of CMD was 93% and the highest severity score was 3 (Table 6). At Rubona, two clones (MM06/0090-OP/12 and MM06/0128-OP/3) out of 12 showed CMD leaf symptoms with severity score 3. For CBSD, three clones (MM06/0128-OP/4, MH05/0091-OP/11 and MH05/0091-OP/12) showed CBSD leaf symptoms at 6 MAP with severity score 3 (Table 7).

Table 6 Incidence and severity of CMD and CBSD at Karama Research Station at 6 MAP (PYT)

No.	Names of clones	CMD incidence (%)	CMD severity	CBSD incidence (%)	CBSD severity
1	Bulk/13	44.8	3	0	1
2	MM06/0128/4	48.0	3	0	1
3	Bulk/16	37.9	3	0	1
4	MM06/0128/1	0	1	0	1
5	MH05/0091/12	12.5	3	0	1
6	MM06/0128/3	93.3	3	33.3	2
7	MM06/0128/10	50.0	3	50	3
8	MH05/0091/11	5.7	2	28.6	3
9	Bulk/35	0	1	0	1
10	MH97/0105 (Check)	8.8	2	100	3
11	MM06/0090/12	69.2	2.5	0	1

Table 7 Incidence and severity of CMD and CBSD at Rubona Research Station at 6 MAP (PYT)

No.	Names of clones	CMD incidence (%)	CMD severity	CBSD incidence (%)	CBSD severity
1	Bulk/13	1.0	3	0	1
2	MM06/0128/4	1.5	3	2	3
3	Bulk/16	4.0	3	0	1
4	MM06/0128/1	0	1	0	1
5	MH05/0091/12	1	3	1	3
6	MM06/0128/3	5.5	3	0	1
7	MM06/0128/10	1	3	0	1
8	MH05/0091/11	0	1	4.5	3
9	Bulk/35	1	3	0	1
10	NASE14 (Check)	0	1	0	1
11	MM06/0090/12	4.3	3	0	1

Reaching farmers directly and through partners

Farmers trained on CMD and CBSD disease symptom recognition and management strategies

Thirty farmers from Bugesera and Ruhango Districts were trained on diagnosis of cassava viral diseases through observation of symptoms, pest and disease scoring, and management options (Figure 10 and Figure 11).

Figure 10 Assessing performance of demonstration plots at Bugesera site (A), farmer weighing NASE14 (B) and CBSD symptoms on variety Ndamirabana used as check (C) in 2017

Figure 11 Assessing performance of demonstration plots at Ruhango site (A), farmer weighing NASE14 (B) and CBSD symptoms on variety Rutanihisha used as check (C) in 2017

Demonstration plots for benefits of using virus-indexed planting materials established on-farm

The demonstration plots were established in Bugesera and Ruhango Districts. At harvesting time in December 2015, data were collected on yield (Table 8 and Table 9). At Bugesera site, farmers appreciated the performance of improved variety, MM98/0105, with yield of 31.6 t/ha compared to the local variety with 9.2 t/ha.

Table 8 Demonstration plot harvest in Bugesera (80 m^2)

Year	Varieties	No. plants harvested	No. roots marketable	No. roots non-marketable	Weight marketable	Weight non-marketable	Total roots	Weight (kg)	Yield (t/ha)
2015	MH98/0105	71	310	160	232	21	470	253	31.6
	Garukunsubire	44	172	100	62	12	272	74	9.2
2017	NASE14	40	174	57	38	4.4	231	42.4	5.3
	Ndamirabana	36	121	145	42.6	14	266	56.6	7.1

Table 9 Demonstration plot harvest in in Ruhango (80m^2)

Year	Varieties	No. plants harvested	No. roots marketable	No. roots non-marketable	Weight marketable	Weight non-marketable	Total roots	Weight (kg)	Yield (t/ha)
2015	MH98/0105	65	210	60	69	27	270	96	13.7
	Cyizere	25	72	50	22	8	122	30	6.3
2017	NASE 14	64	270	135	135	13.5	405	148.5	18.5
	Rutanihisha	34	81	60	41	7.5	141	48.5	6.1

After harvesting, we disseminated the improved variety to 14 farmers from two sites, Nemba and Batima, in Rweru Sector of Bugesera District with 100 cuttings per farmer (Table 10). A total of 1400 cuttings were disseminated. Eight women and six men participated in this event. At Ruhango site, the performance of improved variety MM98/0105 was not good due to susceptibility to CBSD. Its yield was 13.7 t/ha and the local variety was 6.3 t/ha. Due to CBSD susceptibility of the improved variety, the planting materials were not disseminated.

Table 10 Dissemination of cuttings in Rweru Sector of Bugesera District in Rwanda

Sector	Sites	Villages	No. of male	No. of female	No. of cuttings received
Rweru	Nemba	10	6	4	1000
	Batima	4	0	4	400
	Total	**14**	**6**	**8**	**1400**

Information materials developed and disseminated

Training

There were 150 leaflets developed, printed and disseminated to farmers.

Awareness

Two radio talks were done on cassava virus diseases and farmers were free to ask questions about cassava virus diseases.

Participation in agriculture show

To disseminate project information on project activities to different stakeholders promoting cassava production in Rwanda, the team participated in the agriculture show held at Mulindi in June 2016. The stand was visited by farmers, students and agricultural extension agents. During the 10-day period, about 793 show-goers interacted with the team on various subjects including insect pests and diseases affecting cassava, cassava varieties and yields (Figure 12).

Figure 12 CDP-Rwanda team at the agriculture show in Mulindi, June 2016

Build sustainable regional capacity

Project inception and consultative meeting with stakeholders

Two Rwanda Agricultural Board staff including the Country Team Leader (CTL), Ms Marie C. Kanyange, and the Assistant CTL, Mr Gervais Gashaka, participated in the development of the country-specific milestones during the project inception and consultative meeting with stakeholders.

Stakeholder engagement

A number of stakeholders were approached and contact maintained throughout the project. These interested parties ranged from University departments to research institutions and to government departments (see Table 11).

Table 11 Partners and stakeholders visited during the impact assessment baseline study and monitoring and evaluation missions for the 'Disease diagnostics for sustainable cassava productivity in Africa' project, 2014

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
Rwanda Agricultural Board	Rubona, (Southern Province)	NARS (Project partner)	Ms Marie C. Kanyange (CTL) and Mr Gervais Gashaka, (Assis. CTL/Cassava Breeder)	Hosting the project, breeding for resistance to CMD and CBSD and disease diagnostics	Ms Marie C. Kanyange, Mr Gervais Gashaka

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
		NARS (Non-project staff)	Mr Jean Lambert Rurangwa (Quality Seed Control Officer), Mr Jervais Ngererwa (Coordinator of Seed Production Services), Ms Concilie Nyirahorana (Social Economist) and Mr Felix Gatunzi (Research Officer)	Social economics, seed certification and tissue culture	Mr Jean Lambert Rurangwa, Mr Jervais Ngererwa, Ms Concilie Nyirahorana
University of Rwanda	Butare	Universities (stakeholders)	Dr Solange Uwituze (Dean of Faculty of Agriculture), Dr Charles Bucagy (Head of Department, Crop and Horticulture)	Training and supervision of students	Dr Solange Uwituze
NGO-INGABO		NGO	Mr Ntehomvukiye Frav (Agronomist)	Seed multiplication and distribution	Mr Ntehomvukiye Frav
Ituze Farmers' Cooperative	Ituze	Farmers' Cooperative	Mr Sibomana Pascal, (Ituze Cooperative Leader)	Cultivation and processing of cassava	Mr Sibomana Pascal
Kinazi Cassava Processing Plant	Kinazi	Cassava processing facility	Mr Robert Runazi, (Director General)	Processing	Mr Robert Runazi

Exchange visits between scientists in the project countries

Ms Marie C. Kanyange (CTL) and Mr Gervais Gashaka (Assistant CTL) participated in the first exchange visit to Zambia Agriculture Research Institute during 15–21 May 2016. They visited the Cassava Diagnostics Project (CDP) research activities in Zambia including the new screenhouse and laboratories, and farmers' fields to assess the disease situation.

Outreach to regional virologists in non-project countries

Ms Jeanine Umfuyisoni accompanied the TARI–Mikocheni CDP team (Dr J. Ndunguru, Dr P. Sseruwagi and Dr F. Tairo) and Dr Laura Boykin on a fact-finding mission to Madagascar in January 2017. She was instrumental in helping the visiting team in translation of French and English with the hosts in Madagascar. The visit aimed to establish collaboration and exchange experiences on cassava virus diagnostics, field disease assessments and management.

Strengthening human capacity and infrastructure

Human capacity staff in Rwanda

Project staff recruited

Name	Position
Marie C. Kanyange	CTL
Gervais Gashaka	Assistant CTL
Ghislain Niyonteze	Research Assistant
Esperance Munganyinka	PhD student
Jeanine Umfuyisoni	MSc student
Ildephonse Hakizimana	Driver

PhD and MSc training on different aspects of cassava virus diseases:

- One MSc student – Jeanine Umfuyisoni
- Support to MSc research – Concilie Nyirahorana
- One PhD student – Esperance Munganyinka.

Advanced specialized training and visits for project scientists (1–2 months) conducted. During 1–30 June 2015, Marie C. Kanyange and Nurbibi Cossa, Mozambique CTL, visited the Department of Ecology and Evolution and Natural Resources, School of Environmental and Biological Sciences Rutgers University, USA, under the supervision of Professor Siobian Duffy.

The objectives of the visit were:

1. To understand fundamentals of nucleotide sequence alignment
2. To explore phylogenetic relationships as a tool in molecular evolution
3. To go through recombination analysis.

Training	Participants	Number
Training on Disease Diagnostic and Survey Methodologies, 17–24 August 2014	Research assistant and students	3
IP Rights and Communications Nairobi, 26–31 October 2014	CTL and Assistant CTL	2
Data Management, 17–19 February 2015	CTL	1
Rutgers University, for one month (Bioinformatics), 1–30 June 2015	CTL	1
Training of farmers	Farmers	30
Plant and Animal Genome Conference (PAG), AgShare.Today, 9–16 January 2016	CTL and PhD student	2
Financial management training, Mango, Kigali, 2016	CTL and accountant	
Bioinformatics training, MARI, 22–24 February 2016	PhD and MSc students	2
Bioinformatics data analysis, University of Western Australia, 4 July–5 August 2016	MSc student	1
Training on manuscript development and writing by AgShare.Today, Zambia, 3–9 October 2016	PhD and MSc students	2

Infrastructure strengthening

1. Diagnostic and virus-indexing laboratories refurbished
2. CDP project supported cassava program with laboratory equipment including gel documentation, PCR machine and centrifuge
3. Greenhouses constructed/renovated
4. A car was purchased for facilitating project activities.

SECTION THREE: Impacts, success stories and learning outcomes

Impacts

This information was not available at the time of writing.

Success stories

This information was not available at the time of writing.

Learning outcomes

We learned more about team spirit within the region to solve common issues in the agriculture sector, especially concerning cassava viral diseases and networking among regional and international research institutions.

We were able to exchange information on cassava varieties tolerant to both CMD and CBSD that are available in the region.

List of manuscripts

Munganyinka, E., Ateka, E.M., Kihurani, A.W., Kanyange, M.C., Tairo, F., Sseruwagi, P. and Ndunguru, J. (2017) Cassava brown streak disease in Rwanda, the associated viruses and disease phenotypes. *Plant Pathology*, 67:377–387.

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Acknowledgements

This work was supported by Tanzania Agriculture Research Institute (TARI)–Mikocheni with joint funding from the Bill & Melinda Gates Foundation (BMGF) and UK Department for International Development (DFID) through the 'Cassava disease diagnostics for sustainable productivity in Africa' project (Grant no. 51466). We are grateful to Mr Deogratius Mark for technical assistance in laboratory molecular sample analysis.

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MOZAMBIQUE

Nurbibi Cossa[1], Ricardo Marcia [1], Azevedo Surge[1], Agnaldo Alicete[1], Marta Solemanergy[1], Jamissie Amissie[2], Fred Tairo[3], Joseph Ndunguru[3] and Peter Sseruwagi[3]

[1] Mozambique Agrarian Research Institute, Maputo 2698, Mozambique
[2] Mozambique Agricultural Research Institute, Posto Agronómico de Nampula, P.O. Box 622, Rua de corrane, Km 7, Nampula, Mozambique
[3] Tanzania Agricultural Research Institute (TARI)–Mikocheni, P.O. Box 6226, Dar es Salaam, Tanzania

Abstract

The second phase of the Cassava Diagnostics Project (CDP) was implemented in Mozambique from 2013 to 2017. The project addressed three aims: (a) understanding the threat from evolving viruses and vectors, (b) reaching farmers directly and through partners and (c) building sustainable regional capacity. Cassava mosaic disease (CMD), cassava brown streak disease (CBSD) and whitefly abundance were monitored in smallholder cassava fields in seven major cassava growing provinces in the country. The CMD incidence was highest in Gaza province (53.1 %) and Inhambane (51.5%) and lowest in Cabo Delgado (12.1%). There was no significant change in CMD severity among years and provinces. The CBSD incidence was highest in Zambezia (64.5%), followed by Nampula (36.6%) and Cabo Delgado (34.3%) provinces and lowest (<2.0%) in the southern provinces of Inhambane, Gaza and Maputo. Mean CBSD severity score ranged within 2.3–2.6 but with no significant differences among provinces. Generally, adult whitefly abundance was higher in the 2013 and 2015 than the 2017 surveys. Mean whitefly populations were highest in Inhambane (20.26 per plant) and Gaza (18.62) provinces and lowest in Cabo Delgado (3.49) and Nampula (1.34). Molecular characterization showed the presence of two cassava mosaic begomoviruses, namely *African cassava mosaic virus* and *East African cassava mosaic virus*, and two *Cassava brown streak viruses* (CBSVs), CBSV and *Uganda cassava brown streak virus*. The dominant whitefly (*Bemisia tabaci*) population in Mozambique was identified as SSA1-SG3.

Training was conducted for farmers, extension staff and several stakeholders in outreach activities in on-farm demonstration plots in Boane and Inharrime. The outreach activities were conducted on awareness of cassava viral diseases (CMD and CBSD) and their transmission by whitefly vectors and infected planting materials, production and management strategies. In addition, awareness was created the benefits of using virus-free cassava planting materials.

Infrastructure and human capacities to conduct virus diagnostics was greatly improved at Instituto de Investigação Agrária de Moçambique (IIAM), Maputo. The infrastructure included acquisition of diagnostic laboratory equipment, refurbishment of one greenhouse for contained trials, installation of a new water tank and pump to provide steady water supply to the CDP molecular laboratory and other laboratories, and one project vehicle to facilitate transport for field activities. This has not only

enhanced the capacity of IIAM's scientists to do molecular diagnostics, but also other scientists at IIAM, and other institutions in Mozambique. One MSc student and one PhD student were trained.

Acronyms and abbreviations

5CP	New Cassava Varieties and Clean Seed to Combat CBSD and CMD Project
ACMV	*African cassava mosaic virus*
CBSD	Cassava brown streak disease
CBSV	*Cassava brown streak virus*
CDP	Cassava Diagnostics Project
CMB	Cassava mosaic begomovirus
CMD	Cassava mosaic disease
CTL	Country Team Leader
EACMV	*East African cassava mosaic virus*
IIAM	Instituto de Investigação Agrária de Moçambique (Institute of Agricultural Research of Mozambique)
TARI	Tanzania Agricultural Research Institute
UCBSV	*Uganda cassava brown streak virus*
ZARI	Zambia Agricultural Research Institute

Results summary: Mozambique

Aim I: Understand the threat from evolving viruses and vectors	
Objective 1: Disease epidemiology	
Disease and whitefly prevalence surveys conducted	• Three surveys conducted in 2013, 2015 and 2017, covering 1023 fields in seven regions. • Survey data for 2013 and 2015 were cleaned and validated by the TARI–Mikocheni team and Cambridge modeling team during a data entry and assembly workshop held from 27 November to 3 December 2016 in Dar es Salaam, Tanzania. • A new SOP for countywide survey developed and used to collect 2017 data. • Cassava leaf samples with cassava mosaic disease (CMD)- and cassava brown streak disease (CBSD)-like symptoms were collected for laboratory analysis.
Alternative hosts for CBSVs and CMBs and associated insect vectors identified	• Carried out as part of Mr Jamisse Amisse's PhD research. • One manuscript on *Cassava brown streak virus* (CBSV) diversity is currently in press.
Objective 2: Characterization of emerging viruses	
Cassava virus isolates in the project countries sequenced and analyzed	• Seven new whole CBSV genomes from total RNA isolated from CBSD-symptomatic cassava leaves collected from Nampula and Zambezia in Mozambique. • One manuscript by Amisse et al. (2018) 'In press'. Genetic diversity of Cassava brown streak viruses in Mozambique using whole genome sequences, *Plant Pathology.* • Ms Marta Solemanegy characterized the cassava mosaic begomovirus (CMB) isolates from the May 2016 survey in southern provinces of Mozambique using diagnostic PCR species-specific primers. Results revealed the presence of *East African cassava mosaic virus* (EACMV), EACMV-UG2 and *African cassava mosaic virus* (ACMV) in Maputo province. Mixed infections of ACMV and EACMV occurred in all three provinces of the south, except that ACMV was not found in Maputo. *South African cassava mosaic virus* was confirmed to also occur in Mozambique.
Cassava virus distribution maps generated (incidence, severity, whitefly, viruses, sat)	• Disease distribution maps were produced and distributed to the stakeholders.
Objective 3: Characterization of disease vectors	
Potential insect vectors of CBSVs identified	• Carried out as part of Mr Jamisse Amisse's PhD research.
Objective 4: Diagnostic tools	
Lab-based diagnostic tools developed	• Diagnostic primers were developed by Mr Jamisse Amisse and Ms Martha Solemanegy for detection of CBSVs and CMBs, respectively.

Aim II: Support clean seed systems for farmers	
Objective 6: Conventional breeding support	
Breeders' material monitored for disease and indexed for virus (CMBs and CBSVs) load at 3, 6, 9 and 12 MAP	• Twenty-five varieties were indexed for CMBs and CBSVs. • EACMV was detected in 6.98% of the materials assessed. • CBSV was detected in 67% of the materials assessed.
Objective 9: Reaching farmers directly and through partners	
Farmers trained on CMD and CBSD disease symptom recognition and management strategies	• Seventy-two farmers (22 male and 50 female) were trained at the demonstration sites in 2013 and 2015.
Demonstration plots for benefits of using virus-indexed planting materials established on-farm	• Two demonstration plots were set up to show farmers the benefits of clean planting materials.
Information materials developed and disseminated	• One article was published 1 April 2015 in Canal de Mozambique on cassava viral diseases and their management. • One pamphlet on identification and management of cassava viral diseases.
Aim III: Build sustainable regional capacity	
Objective 10: Strengthening stakeholder linkages	
Team leader meeting to develop country-specific milestones	• One Country Team Leader (CTL) and one assistant participated in the development of country-specific milestones.
Project inception and consultative meeting with stakeholders conducted	• One CTL and one assistant participated in project inception and consultative meetings.
Awareness on availability of diagnostic capacities created through training and different media	• One radio broadcast aired on different topics on cassava virus diseases and management. • One article published in Agritech News. • One brochure developed (>1500 copies issued).
Exchange visits between scientists in the project countries conducted	• The Instituto de Investigação Agrária de Moçambique (IIAM) Cassava Diagnostics Project (CDP) team leader, Ms Nurbibi Coosa and her assistant participated in the first exchange visit to Zambia Agricultural Research Institute during 15–21 May 2016. • The team visited the CDP research activities in Zambia, including the new screenhouse and laboratories, and farmers' fields to assess the disease situation.

Objective 11: Strengthening human capacity and infrastructure	
Human capacity	
Project staff recruited	• Seven staff: one driver, one MSc and one PhD student, two research assistants, one assistant CTL and one CTL.
PhD and MSc students trained on various aspects of cassava virus diseases	• One PhD and one MSc student trained.
Advanced specialized training and visits for project scientists (1-2 months) conducted	• The CTL, Ms Nurbibi Cossa, visited the Agricultural Research Organisation of Israel during 2–8 February 2014 with TARI–Mikocheni project management and all six CTLs. • Ms Nurbibi Cossa visited Rutgers University, USA, to learn analysis of begomoviruses in Mozambique in June 2015 with instruction from Prof Siobain Duffy.
Extension workers, crop inspectors and other stakeholders (1 week) training	• One workshop conducted for extension officers.
Project staff trained on IP, biosafety issues and communication strategies	• Ms Nurbibi Cossa attended training in intellectual property and communication strategies for 17 project scientists during 27–31 October 2014 at ICRAF, Nairobi, Kenya.
Project results and information disseminated	• Two journal articles accepted in Physiological and Molecular Plant Pathology (Non-cassava host plants with CBSVs) and Plant Pathology (CBSVs diversity).
Institute Directors trained in Leadership and management	• Director of IIAM, Mozambique, attended the 'Leadership Skills for Institutional Directors' conducted in July 2014 in Entebbe, Uganda, and Kigali, Rwanda.
Infrastructure strengthening	
Greenhouses constructed/renovated	• One greenhouse renovated and in use. • One water tank to supply the molecular laboratory.
Vehicles, laboratory equipment and consumables procured	• One project vehicle procured and in use. • Various equipment procured and in use.
Project management	
Project management	• One IIAM Accountant participated in the workshop on accounting package TALLY, during 14–17 May 2013 in Dar es Salaam, Tanzania. • The CDP CTL, Ms Nurbibi Cossa, participated in the financial management training workshop in Kigali, Rwanda, in 2015. • The CTL and students attended the AgShare.Today training and scientific report writing skills in January 2016 in San Diego, USA.

Background

Cassava (*Manihot esculenta* Crantz.) was introduced to Mozambique by the Portuguese in the 17th century (Thresh and Hillocks, 2003). Cassava is one of the most important food crops in the country. It ranks fourth as a food staple after maize with 5.3 million tonnes produced annually (FAO, 2014). Estimates from FAO for Mozambique indicate that over 80% of cassava is consumed as food. In terms of regional distribution of consumption, cassava is a major food staple in central and northern Mozambique and is mainly consumed roasted and in the form of flour mixed with water to make nutritious porridge. Fried or boiled fresh cassava roots constitute almost 12% of the total cassava consumed in the southern region of the country (IITA, 2008; Dias, 2012).

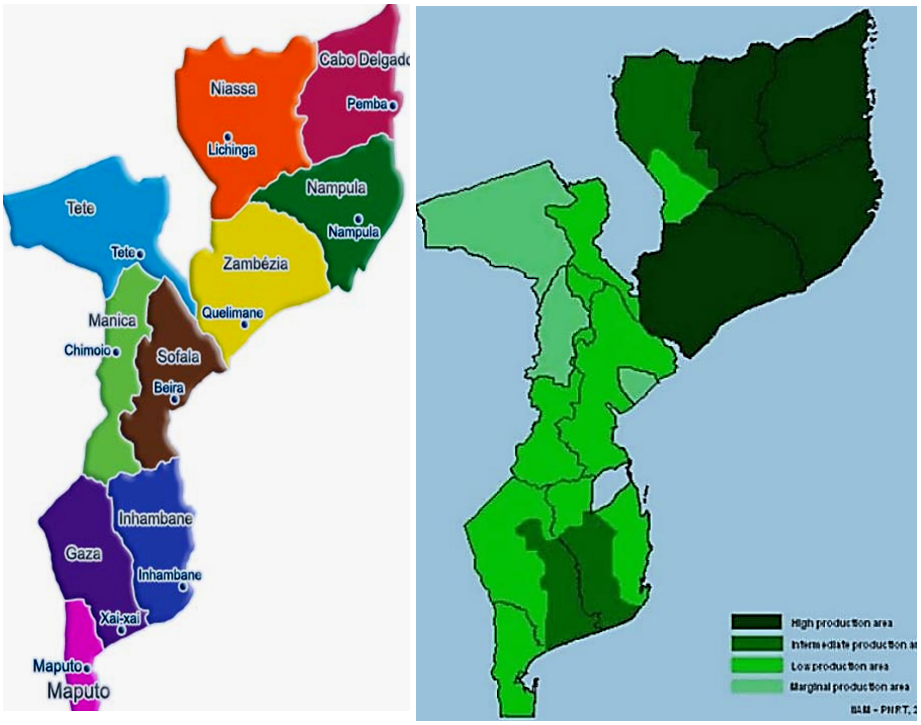

Figure 1 Cassava production areas in Mozambique; Source: IIAM/PNRT (2007)

Cassava is produced countrywide, although the highest production area is in the northern provinces of Nampula, Niassa and Cabo Delgado, in the central province of Zambezia and the southern provinces of Inhambane, Gaza and Maputo (Figure 1). Production is mainly by small-scale farmers, where roughly 61% are engaged in production of the crop on marginal and sub-marginal lands (IITA, 2008; Dias, 2012). Production reached a maximum of 10.09 million tonnes in 2011 compared to 5.3 million tonnes in 2014. Yields followed the same pattern and reached a maximum of 78.02 hg/ha in 2011 compared to 60.9 hg/ha in 2014, which is however still below the average of most African countries (9.3 t/ha). Under ideal growing conditions, disease-free cassava can yield more than 30 t/ha. However, despite attributes such as drought tolerance and low input requirements, yield is poor with only 4 t/ha. This low yield can be due to both abiotic and biotic stresses. The most important of the biotic factors are thought to be pests and diseases. Poor agronomic practices, lack of clean planting materials, low yielding varieties and long maturity periods are additional major problems (IITA, 2008; Dias, 2012). Of the diseases that affect cassava production, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) contribute the most to yield loss (Cossa, 2005). Because of these two diseases, many farmers have turned to alternative crops such as maize and sweet potato to ensure food security in their households. The CBSD is reported to occur mainly

in the coastal regions of northern Mozambique (Hillocks et al., 2002). In contrast, CMD occurs all over the country where cassava is grown. At least five cassava mosaic begomovirus (CMB) species were reported to cause CMD in Mozambique: *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), *East African cassava mosaic Malawi virus*, *East African cassava mosaic Cameroon virus* and *South African cassava mosaic virus* (Cossa, 2005).

SECTION ONE: Understanding the threat from evolving viruses and vectors

Disease epidemiology

Sampling framework and data collection

Countrywide cassava virus disease monitoring surveys were carried out in 2013, 2015 and 2017 in seven provinces: Cabo Delgado, Gaza, Inhambane, Niassa, Maputo, Nampula and Zambezia. The main aim of the countrywide surveys was to understand the threat from the evolving virus and vectors in Mozambique, with specific focus on determining the status of CMD and CBSD and characterizing the associated insect vectors and mapping the geographical occurrence of these two major diseases in Mozambique.

During surveys, cassava fields were sampled along major roads (Figure 2) and representative samples collected according to Sseruwagi et al. (2004) and the modified Cassava Diagnostics Project (CDP) harmonized standard operating procedure protocol (CMD/CBSD survey manual on the CDP intranet on the AgShare.Today platform; Sseruwagi et al., 2017).

Figure 2 (left) Scientists assessing cassava viral diseases and insect vectors with smallholder farmer and (right) scientists assembling leaf samples for safe transportation and storage until laboratory analysis

Results from the countrywide survey showed CMD occurred in all provinces. Incidence was generally moderate, and the trend showed that CMD incidence decreased from 2013 to 2017 (Table 1); however, disease severity increased. Across provinces, the most affected province was Gaza with mean CMD incidence of 53% followed by Inhambane with 51% and the least was Cabo Delgado with 14%.

The CMD symptom severity varied between years with the trend showing no significant change between years. The highest CMD severity score was in 2017 (3.04) and the lowest was in 2015 at (2.16). Among the seven provinces, Maputo had a slightly elevated severity score (Table 1, Figure 3 and Figure 4).

Table 1 Incidence (Inc.) and mean symptom severity (sev.) of CMD in major cassava growing provinces in Mozambique (2013–2017). '–' indicates that the district was not surveyed during those years

Province	2013		2015		2017			
	Inc. (%)	Mean sev.	Inc. (%)	Mean sev.	Inc. (%)	Mean sev.	Overall mean inc. (%)	Overall mean sev.
Cabo Delgado	20.8	2.5	5.3	2.1	10.2	2.88	**12.1**	**2.5**
Inhambane	81	3	37.02	2.28	36.37	3.06	**51.5**	**2.8**
Gaza	75	2.9	33.3	2.1	51.01	3.31	**53.1**	**2.8**
Maputo	38.1	2.9	20.27	2.2	35.56	3.08	**31.3**	**2.7**
Nampula	17	2.4	1.5	2.04	8.47	2.88	**9.00**	**2.4**
Niassa	29	2.6	–	–	–	–	–	–
Zambezia	36	2.5	18.7	2.22	20.73	3.0	**25.1**	**2.6**
Mean	**42.41**	**2.69**	**19.35**	**2.16**	**27.06**	**3.04**	**30.35**	**2.63**

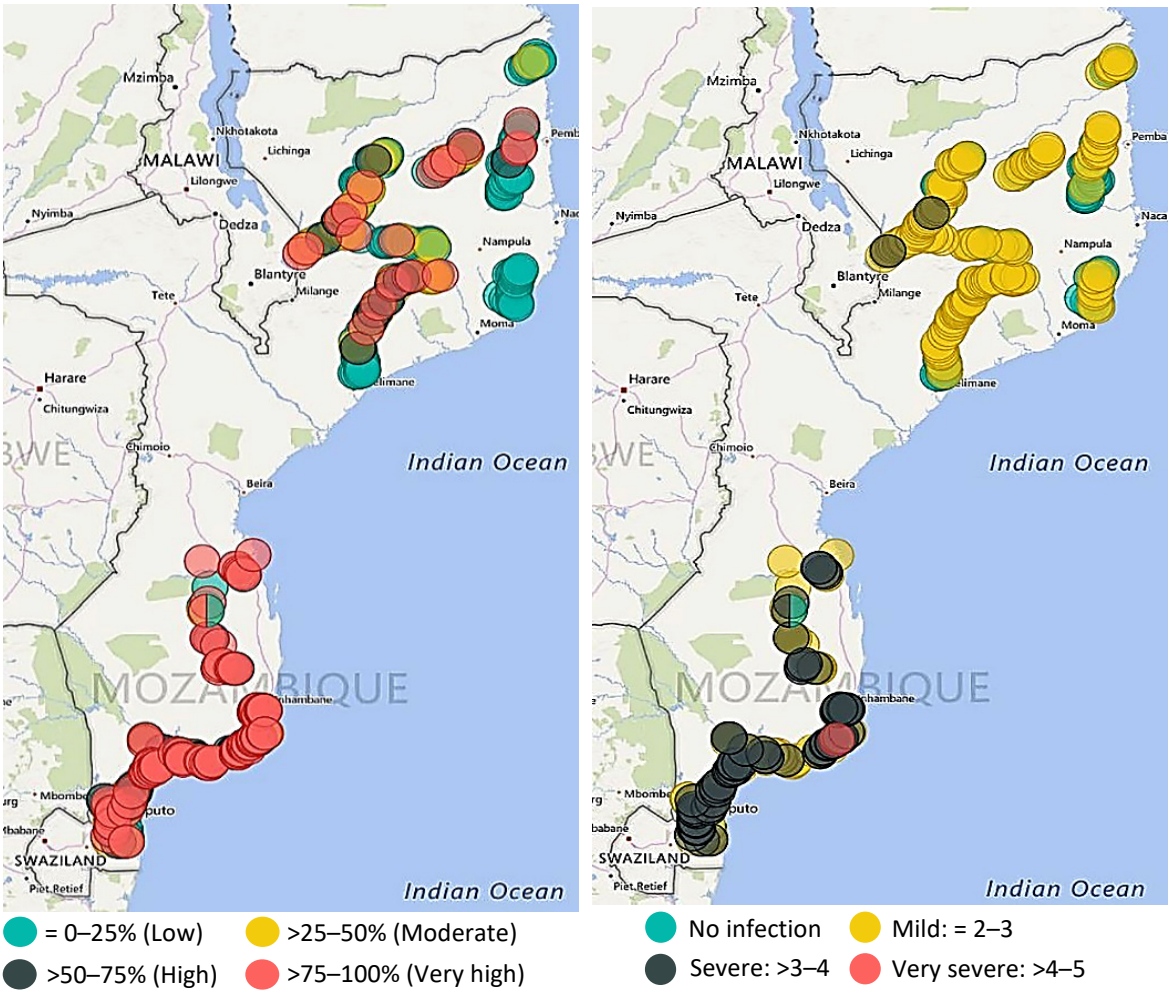

Figure 3 Distribution and severity of CMD – 2013 survey: (left) incidence and (right) severity

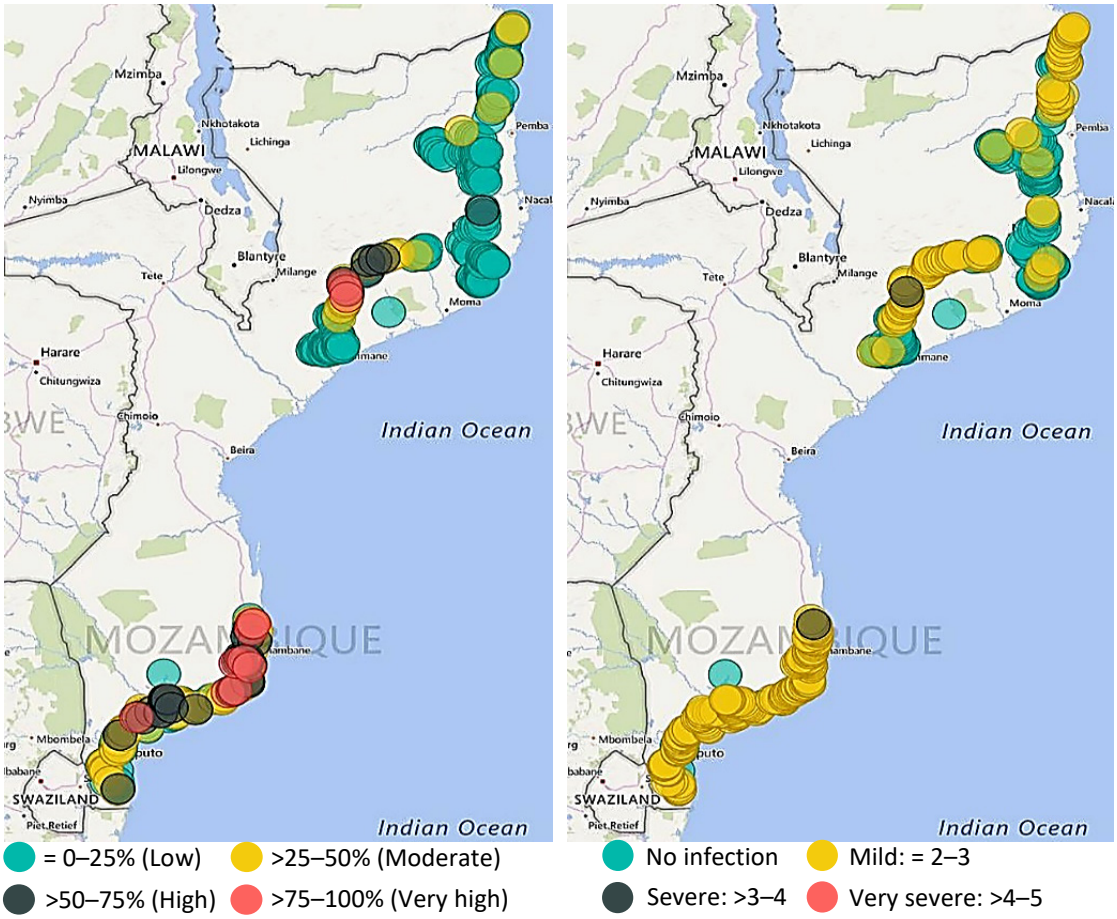

Figure 4 Distribution and severity of CMD – 2015 survey: (left) incidence and (right) severity

Incidence and severity of CBSD

An increasing trend of CBSD incidence was observed during 2013–2017. The most infected province was Zambezia during those years (Table 2). Unlike CMD, CBSD was recorded in few provinces; three provinces – Gaza, Inhambane and Maputo – had incidences of CBSD <5% in 2013. No CBSD was noted in these provinces for 2015 and 2017 surveys (Table 2, and Figure 5 and Figure 6).

Table 2 Mean incidence (Inc.) and mean symptom severity (sev.) of CBSD in major cassava growing provinces in Mozambique (2013–2017). '–' indicates that the district was not surveyed during those years

Province	2013		2015		2017			
	Inc. (%)	Mean sev.	Inc. (%)	Mean sev.	Inc. (%)	Mean sev.	Overall mean inc. (%)	Overall mean sev.
Cabo Delgado	14.5	2.7	17.5	2.6	70.78	2.66	**34.3**	**2.65**
Inhambane	0.46	2.1	0	0	0	0	**0.2**	**2.10**
Gaza	0.46	2.1	0	0	0	0	**0.2**	**2.10**
Maputo	4.35	2.2	0	0	0	0	**1.5**	**2.20**
Nampula	20.73	2.9	13.47	2.1	75.7	2.54	**36.6**	**2.51**
Niassa	1.9	2.7	–	–	–	–	–	–
Zambezia	67.9	2.7	60.79	2.2	64.69	2.65	**64.5**	**2.52**
Mean	**15.76**	**2.49**	**15.29**	**2.3**	**35.20**	**2.61**	**22.9**	**2.35**

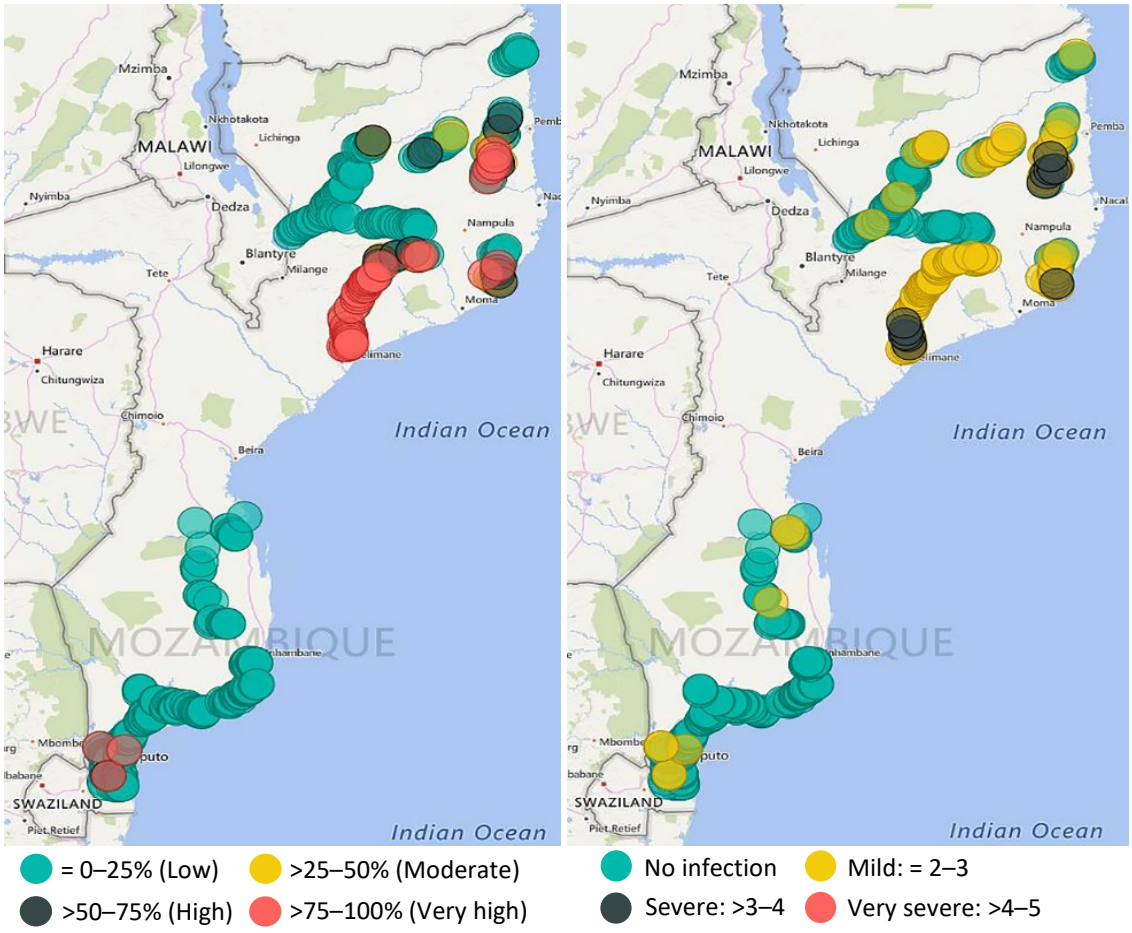

Figure 5 Distribution and severity of CBSD – 2013 survey: (left) incidence and (right) severity

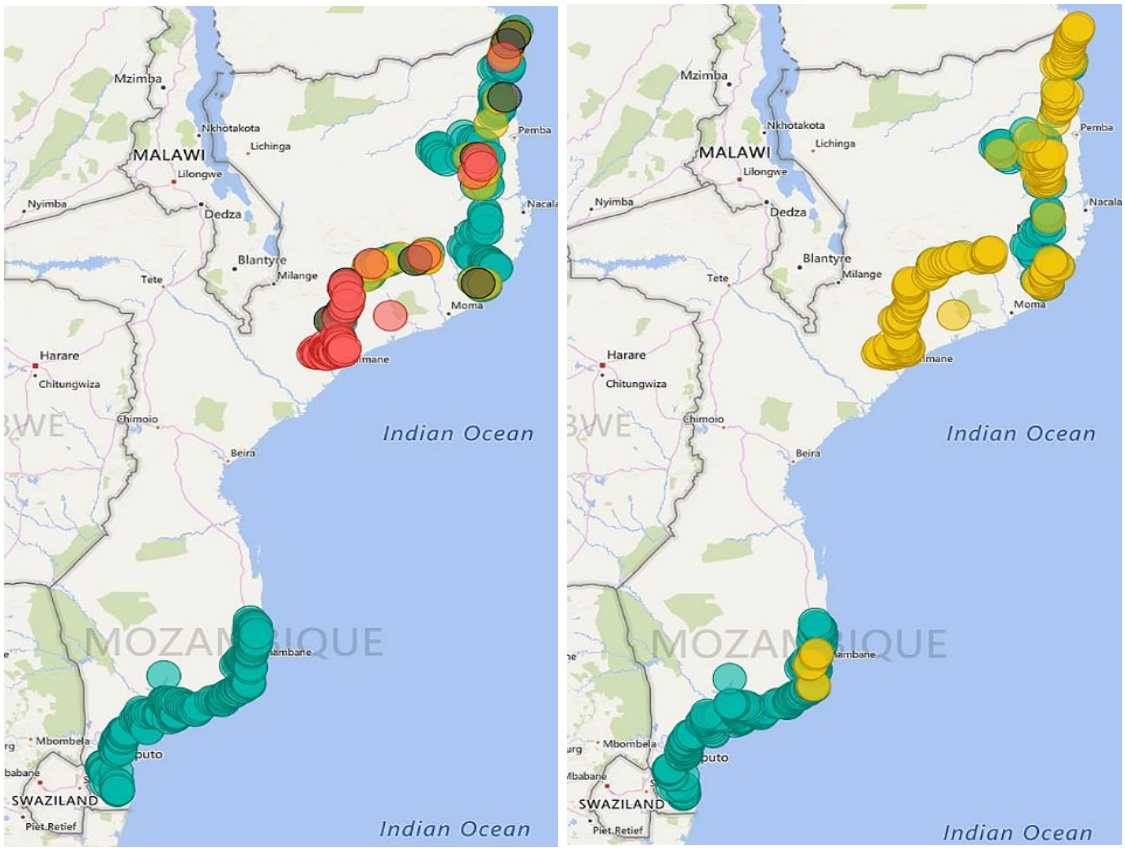

Figure 6 Distribution and severity of CBSD – 2015 survey: (left) incidence and (right) severity

The CBSD symptom severity was moderate across years and regions. Mean severity score ranged within 2.3–2.6, although there was no difference in CBSD severity between regions.

Adult whitefly abundance

Whitefly numbers were low to moderate in all surveys, with Inhambane showing slightly higher whitefly counts per plant (20.26) in the three surveys (Table 3), and the least was Maputo (1.34). Across surveys, the highest mean whitefly count was 12.02 per plant in 2013 compared to 2017 with 3.45 per plant (Table 3).

Table 3 Mean whitefly counts per plant in major cassava producing provinces in Mozambique (2013–2017). '–' indicates that the province was not surveyed during those years

Province	2013	2015	2017	Mean
Cabo Delgado	2.4	3.71	4.56	**3.49**
Inhambane	33.02	22.98	4.8	**20.26**
Gaza	29.8	18.18	7.88	**18.62**
Maputo	0.59	9.8	1.01	**3.8**
Nampula	0.8	1.4	0.98	**1.34**
Niassa	–	–	–	
Zambezia	5.52	10.57	1.48	**6.36**
Mean	**12.02**	**11.11**	**3.45**	

Alternative hosts for CBSVs and associated insect vectors

This work was addressed by Mr Jamisse Amisse as part of his PhD research. It aimed to determine the alternative hosts for *Cassava brown streak viruses* (CBSVs). Using transmission studies, all insects found on CBSD-infected plants in cassava fields were collected, sequenced characterized and used for transmission studies to see if they played a role in transmitting CBSVs.

Characterization of emerging viruses

CMD- and CBSD-symptomatic leaf samples were collected in Inhambane, Gaza and provinces in the 2015 countrywide survey. The samples were analyzed using PCR. The results showed the presence of two CMBs (ACMV and EACMV) and two CBSVs (CBSV and *Uganda cassava brown streak virus* – UCBSV).

Characterization of disease vectors

A total of 1800 whitefly samples were collected from six provinces in Mozambique during the 2013, 2015 and 2017 surveys (Table 4). DNA was extracted from all samples and sequence-characterized using mtCO1 markers in order to determine the identity of whitefly species prevalent in Mozambique that may vector CMD and CBSD.

DNA was extracted from 508 samples of which 98 were successfully amplified by the *mtCO1* primer pair. A total of 45 DNAs of the 202 samples were submitted for sequencing at Fasteris SA, Switzerland, and the sequences sent to TARI–Mikocheni for analysis by Dr Peter Sseruwagi to be included in a regional manuscript on *B. tabaci*. The dominant *B. tabaci* species in Mozambique was identified as SSA1-SG3.

Table 4 Whiteflies amplified and sequenced from various regions of Mozambique

Survey	No. of samples collected	Samples amplified	No. of samples sequenced
2013	415	21	0
2015	683	98	45
2017	702	83	0
Total	**1800**	**202**	**45**

SECTION TWO: Integrated pest management

Conventional breeding support

Breeders' material monitored for disease and indexed for viruses (CMBs and CBSVs)

To ensure effective breeding for durable resistant cassava varieties in Mozambique, the CDP team supported breeding of resistant cassava materials through virus indexing and breeder trial joint monitoring and virus load evaluation on promising materials and potential parents. In 2013, five variety trials were assessed for cassava viruses (three in Nampula and two in Umbeluzi) (Table 5); and in 2015, one multiplication plot of 25 varieties for the New Cassava Varieties and Clean Seed to Combat CBSD and CMD Project (5CP) was monitored for virus response at Umbeluzi, and 10 in Nampula and 46 representative samples evaluated for virus presence (Table 6).

Table 5 Detection of CMBs in samples from breeders of Mozambique in 2013

Region	No. of samples tested	EACMV	ACMV	EACMV + ACMV
Mongicual	43	6.98 %	0	0
5CP Boane	10	0	0	0
Total	**53**	6.98 %	**0**	**0**

Table 6 Detection of CBSVs in samples from breeders of Mozambique in 2015

Region	No. of samples tested	UCBSV	CBSV	CBSV + UCBSV
Mongicual	43	0	0	0
5CP Boane	29	0	0	0
5CP Nampula	18	0	16.67 %	0
Total	**90**	**0**	**16.67%**	**0**

Reaching farmers directly and through partners

Farmers trained on CMD and CBSD disease symptom recognition and management strategies

This activity aimed at creating awareness of CMD and CBSD among cassava farmers in Coast, Western, Eastern and Nyanza regions. All farmers that were visited during the survey were 'trained' on identification (symptom recognition) and management of the diseases and the benefits of using virus-free materials. On-farm demonstration plots and virus-free cassava multiplication plots were established in two regions: Boane and Inharrime.

Two trainings for farmers and extension workers were conducted respectively at Umbeluzi and Nhacoongo. A total of 72 farmers (22 men and 50 women) were trained in the two regions.

Theoretical background on the concepts of what diseases are, what causes them, etiology and symptomatology of CMD and CBSD were introduced. Groups were formed to discuss control strategies for the diseases. The training also included field practicals, to teach trainees how to recognize and identify CMD and CBSD in the field, and how to identify the whitefly vector.

Two demonstration plots were established at Boane and Nhacoongo districts (Figure 7), to create awareness among farmers on CMD and CBSD, and to show the benefit of using clean plant materials. During surveys, farmers were also taught about the diseases and about roguing infected plants.

Demonstration plots for benefits of using virus-indexed planting materials established on-farm

Demonstration plots were established at Boane and Nhacoongo districts (Figure 7 and Figure 8), to create awareness among farmers on CMD and CBSD, and to show the benefit of using clean plant materials. During surveys, farmers were also taught about the diseases and about roguing infected plants.

Figure 7 Two demonstration plots established at Boane and Nhacoongo districts, to create awareness among farmers on CMD and CBSD diseases and the benefit of using clean planting materials

Figure 8 (A) Demonstration plot establishment in Nhacoongo in 2017, (B) demonstration plot established in Boane district in 2015, (C) demonstration plot in Nhacoongo in 2015, (D) distribution of clean planting material to farmers in Boane district in 2017

Information materials developed and disseminated

A pamphlet (Figure 9) concerning identification and management of CMD and CBSD was distributed to farmers, extension workers and inspectors.

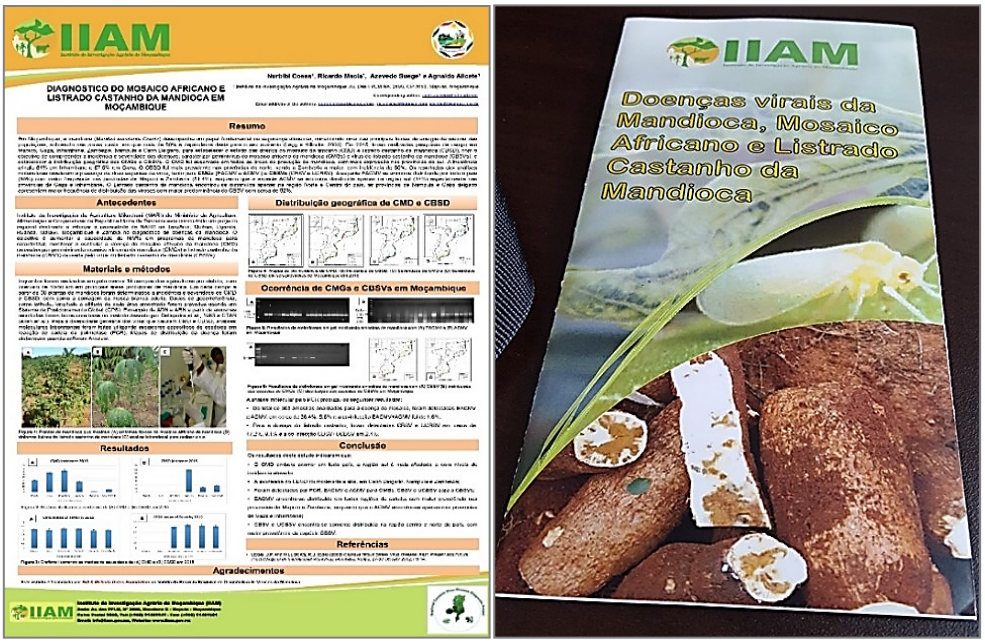

Figure 9 Pamphlet on identification and management of CMD and CBSD

A journal article was published in Canal de Mozambique Journal (Figure 10) about the issue of cassava viral diseases in Mozambique, and the steps taken to minimize the impacts of CMD and CBSD.

1 April 2015 ·

Doenças virais ameaçam cultura da mandioca no país (#canalmoz)

Maputo (Canalmoz) – Doenças provocadas por vírus, nomeadamente, a mosaico africano da mandioca e a podridão radicular, estão a comprometer os níveis de produção da mandioca no país.

Segundo Nurbibi Cossa, do Instituto Nacional de Investigação Agrária de Moçambique, as doenças, registadas pela primeira em 1999 na zona norte país, estão neste momento a propagar-se para a zona sul.

Segundo Nurbibi Cossa, a mosaico africano da mandioca e podridão radicular são doenças que, quando atacam, as plantas da mandiocaficam com as folhas amareladas e atrofiadas, e por causa disso a sua raiz não se desenvolve, razão pela qual são consideradas principais limitantes da produção da mandioca.

Com a propagação em quase todo o território nacional, os níveis de produção deste produto agrícola mais consumido no país depois do milho, baixaram drasticamente, chegando a atingir 70% de redução na produção global em Moçambique.

Estação Agrária do Umbelúzi ensaia mandioca resistente a doenças virais

Por se tratar de doenças sem tratamento, uma vez que ainda não foram descobertos insecticidas, o IIAM, está neste momento a fazer ensaios de variedades de mandioca melhorada, na Estação Agrária do Umbelúzi, com vista a testar a resistências às referidas doenças.

Este trabalho está a ser desenvolvido em quase todas as províncias com elevado potencial de produção de mandioca, como é o caso de Inhambane e de Nampula.

Caso sejam aprovadas, as referidas variedades serão multiplicadas e distribuídas pelos produtores, para que possam recuperar os níveis de produção e de produtividade da mandioca.

Paralelamente aos trabalhos de ensaios de novas variedades da mandioca resistentes a doenças que limitam a produção da mandioca no país, o IIAM lançou na terça-feira, na Estação Agrária do Umbelúzi, no distrito de Boane, província de Maputo, um programa nacional de formação de produtores de mandioca do sector familiar em matéria de identificação e vigilância das referidas epidemias. (Raimundo Moiane

TRANSLATED FROM PORTUGUESE

1 April 2015

Viral diseases threaten the country's cassava crop.

Maputo (Canalmoz) - Diseases caused by viruses, namely the African cassava mosaic and root rot, are jeopardizing the levels of cassava production in the country.

According to Nurbibi Cossa, of the National Mozambique Agricultural Research Institute, the diseases were detected for the first time in 1999 in the north of the country and are now spreading to the south.

According to Nurbibi Cossa, the African cassava mosaic and root rot are diseases that attack cassava plants resulting in yellowish and atrophied leaves. Because of this their roots do not develop and this is the main factor limiting cassava production.

With spread to almost all the nation, the production levels of the country's most consumed agricultural product after maize have fallen drastically by 70%.

The Umbeluizi Agricultural Research Station researches cassava resistant to viral diseases.

Due to the fact that these diseases are not treated and no effective insecticide has been discovered, the IIAM is carrying out trials of improved cassava varieties at the Umbeluizi Agricultural Research Station to test the resistance to these diseases.

This work is being carried out in almost all provinces with high potential for cassava production, such as Inhabane and Nampula.

If approved, these varieties will be multiplied and distributed by producers so that they can recover the production and productivity levels of cassava.

In parallel with the testing of new disease-resistant cassava varieties to increase cassava production in the country, IIAM launched a national training program for cassava producers in the country at the Umbeluizi Agricultural Research Station in the Boane district of Maputo on Tuesday – the aim of the program is the identification and surveillance of these epidemics (Raimundo Moiane).

Figure 10 Canal Moz article on cassava viral diseases and their management

Awareness

A radio broadcast was aired on different topics related to cassava virus diseases and management. Additionally, one article was published in Agritech News, and one brochure was produced and over 1500 copies were distributed.

Strengthening stakeholder linkages

As a way of building sustainable national capacity in disease diagnostics, Instituto de Investigação Agrária de Moçambique (IIAM) strengthened its linkages with stakeholders by enhancing the accessibility of information and technologies to farmers through stakeholders. Thus, for the past four years, IIAM has worked with several stakeholders (Table 7) to enhance accessibility of information to farmers.

Table 7 Stakeholders collaborating with IIAM

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
Institute of Agricultural Research of Mozambique (IIAM)	Maputo	NARS (Project partners)	Ms Nurbibi Cossa (Team Leader), Mr Azevedo Suege (Research Assistant), Mr Agnaldo Alicete (Research Technician)	Hosting the project, breeding for resistance to CMD and CBSD and disease diagnostics	Ms Nurbibi Cossa Mr Azevedo Suege
IIAM	Maputo	Project Students	Mr Jamisse Amisse, Miss Martha Solemanegy	Research and cassava virus diagnostics	Mr Jamisse Amisse
IIAM	Maputo	NARS (Plant protection, biotechnology and seed certification)	Ms Serafina Ernesto Mangana (Head of Plant Protection Department), Mr Frederico Pedro Madabula (Biotechnologist), Mr Elsa Timana (Head of Department, Seed Certification)	Plant health, biotechnology research and seed certification	Ms Serafina Mangana Mr Frederico Madabula Mr Elsa Timana
Ministry of Agriculture, National Directorate of Rural extension	Maputo	Government	Mr Leitao Pedro Isabel (Program Officer/Extensionist)	Training farmers, distribution of planting materials	Mr Leitao Pedro Isabel

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
Service of Economic Activities in Boane district	Boane	Government	Ms Carolina Sitoe (Extensionist)	Training of farmers and distributing clean planting materials	Ms Carolina Sitoe
Farmers	Saldanha, Boane district	Farmer	Ms Amelia Sitoe, Mr Elias Matola, Mr Francisco Matola and Mr Eugenio Freira	Cassava production	Mr Francisco Matola
Eduardo Mondlane University	Maputo	University	Mr Amandio Miguel Muthambe (Plant Pathologist and Course Dean)	Training and supervision of students	Mr Amandio Miguel Muthambe

Exchange visits between scientists in the project countries

The CTL, Ms Nurbibi Cossa, and her assistant participated in the first exchange visit to the Zambia Agricultural Research Institute (ZARI) during 15–21 May 2016. They visited the CDP research activities in Zambia including the new screenhouse and laboratories, and farmers' fields to assess the disease situation.

Strengthening human capacity and infrastructure

During the project duration, several staff provided the project with a range of skills (Table 8).

Table 8 Project staff

S/No.	Name	Position
1	Nurbibi Cossa	CTL
2	Ricardo Macia	Assistant CTL
3	Azevedo Suege	Research Assistant
4	Agnaldo Alicete	Project Technician
5	Artur Olisses	Project Driver
6	Jamisse Amisse	PhD student (Makerere University)
7	Marta Solemanigy	MSc student (Makerere University)

Figure 11 Project staff trained in field disease assessment and laboratory analysis by Dr Peter Sseruwagi and Ms Catherine Gwandu from TARI–Mikocheni, Tanzania, June 2013

Table 9 Summary of short-term training conducted for capacity building to TARI–Mikocheni staff for the period of 2009–2017

Professional training	Year	Venue
Virus diagnostics and laboratory practices	2009/2013	TARI–Mikocheni Biotech Lab-DSM, Tanzania
Scientific writing skills	2010/ 2016	Mombasa, Kenya; San Diego, USA and Lusaka, Zambia
Training in IP	2014	Icipe, Nairobi, Kenya
Cassava disease diagnostics and survey methodologies	2014	Dar es Salaam, Tanzania
Advanced specialized training and visits for project scientists (1–2 months) conducted	2015	Department of Ecology and Evolution and Natural Resources, Rutgers University, USA
Development and training in use of a TARI–Mikocheni CDP SharePoint system	2015	Dar es Salaam
Workshop on bioinformatics	2016	TARI–Mikocheni, Dar es Salaam
PAG 2016	2016	San Diego, USA
Women in Science Leadership	2016	San Diego
Data sharing	2016	Dar es Salaam
Exchange visits between scientists in the project countries	2016	ZARI, Lusaka, Zambia

Infrastructure strengthening

From 2013 until 2016 the infrastructure of IIAM was significantly enhanced through acquisition of new basic equipment for virus diagnostics. The procured infrastructure and status are listed in Table 10.

Table 10 Equipment procured under CDP

Asset-description	Serial no.	Date of purchase	Current location	Condition
Toyota Hilux 4WD Double cabin	ADF-108-MP	2013	IIAM	Good
Generator BUNDU POWER	BP20S3, 20KVA	2013	IIAM	Good

Asset-description	Serial no.	Date of purchase	Current location	Condition
Greenhouse rehabilitation		2013–2017	IIAM	Good
Weighing balance	419641/14	2014	IIAM	Good
Microwave oven	DEFY-DMO349	2013	IIAM	Good
ELISA reader	PR4100	2014	IIAM	Good
BioDoc-It imaging system	UVP trans illuminator	2014	IIAM	Good
PCR machine ɤ TECHNE	53897-2	June 2016	IIAM	Good
Freezer ultra –85°C	Chest, 130 L	June 2016	IIAM	Good
Freezer –20°C and 4°C	Samsung	2016	IIAM	Good
Desktop HP	Intel(R)Pentium® CPU-G3250@3.20GHz 4.00 GB RAM	2016	IIAM	Good
Printer HP	HP LaserJet P1102	2016	IIAM	Good
Gilson pipettes	P200, P20 and P10	June, 2016	IIAM	Good
Stabilizer	6313A01284	2014	IIAM	Good
Tablet	Samsung Galaxy Tab A6	2017	IIAM	Good

SECTION THREE: Impacts, success stories and learning outcomes

Impacts

This information was not available at the time of writing.

Success stories

This information was not available at the time of writing.

Learning outcomes

We learned more about team spirit within the region to solve common issues in the agriculture sector, especially cassava viral diseases and networking among regional and international research institutions.

We have been able to exchange information on cassava varieties tolerant to both CMD and CBSD and that are available in the region.

List of manuscripts

Amisse, J.G., Ndunguru, J., Tairo, F., Ateka, E., Boykin, L.M., Kehoe, M.A., Cossa, N., Rey, C.M. and Sseruwagi, P. (2018) Analyses of seven new whole genome sequences of cassava brown streak viruses in Mozambique reveals two distinct clades: evidence for new species. In Press. *Plant Pathology*.

Amisse, J.G., Ndunguru, J., Tairo, F., Boykin, L.M., Kehoe, M.A., Cossa, N., Ateka, E., Rey, C.M. and Sseruwagi, P. (2018) First report of Cassava brown streak viruses on alternative hosts in Mozambique. *Physiological and Molecular Plant Pathology,* https://doi.org/10.1016/j.pmpp.2018.10.005

Acknowledgements

This work was supported by the Tanzania Agricultural Research Institute (TARI)–Mikocheni with joint funding from the Bill & Melinda Gates Foundation (BMGF) and UK Department for International Development (DFID) through the 'Cassava disease diagnostics for sustainable productivity in Africa' project (Grant no. 51466). We are grateful to Ms Catherine Gwandu and Mr Deogratius Mark for technical assistance in laboratory molecular sample analysis.

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Hillocks, R.J., Thresh, J.M., Tomas, J., Botao, M., Macia, R. and Zavier, R. (2002) Cassava brown streak in northern Mozambique. *International Journal of Pest Management*, 48:179–182.

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Sseruwagi, P., Tairo, F., Stutt, R., Szyniszewska, A. and Godding, D. (2017) Cassava virus and whitefly surveillance: standard operating procedure, January 2017. https://docs.google.com/ document/d/19YtZcI7_k-FvTrdsJvz5yTqOlXMhqb83YYRLuV 7tzDY/ edit?usp=sharing.

Thresh, J.M. and Hillocks, R.J. (2003) Cassava mosaic and cassava brown streak diseases in Nampula and Zambézia provinces of Mozambique. Roots 9 (Forthcoming).

MALAWI

Willard Mbewe[1], Albert Mhone[1], Ruth Semu[1], Michael Benjala[1], Andrew Mtonga[1], Ibrahim Benesi[1], Pilirani Pankomera[1], Joseph Ndunguru[2], Peter Sseruwagi[2] and Fred Tairo[2]

[1] Department of Agricultural Research Services (DARS), Chitedze Agricultural Research Station, P.O. Box 158, Lilongwe, Malawi
[2] Tanzania Agriculture Research Institute (TARI)–Mikocheni, P.O. Box 6226, Dar es Salaam, Tanzania

Abstract

In the past 10 years (2008–2018) the Department of Agricultural Research Service at Chitedze Research Station has implemented the Cassava Diagnostics Project (CDP). The project had three main aims to address cassava diseases: (i) understanding the threat from the evolving virus and vectors, (ii) contributing to an integrated pest management system and (iii) building sustainable regional management. Three country-wide epidemiological surveys were carried out in 2009, 2013 and 2015 to understand the genetic diversity of Cassava mosaic begomoviruses (CMBs) and Cassava brown streak ipomoviruses – *Cassava brown streak virus* (CBSV) and *Uganda cassava brown streak virus* (UCBSV) – affecting cassava, and to establish the evolutionary relationship of the viruses with the aim of improving diagnostics and disease management, and consequently enhancing food security in Malawi.

The results of the three surveys showed a decrease in levels of cassava mosaic disease (CMD) infections recorded in 2009, 2013 and 2015. The CMD incidence was highest in southern regions (45.7%) and lowest in the northern regions (20.6%). Mean CMD symptom severity was 2.4. However, cassava brown streak disease (CBSD) incidence was higher in the northern regions bordering Tanzania (31.5%) than the southern regions (8.5%). Mean CBSD severity was 2.8. Genetic diversity analysis using both Sanger and Illumina deep sequencing techniques revealed existence of three CMB species in Malawi: *East African cassava mosaic virus* (EACMV), *East African cassava mosaic Malawi virus* (EACMMV) and *East African cassava mosaic Zanzibar virus*. Recombinant CMB isolates included EACMV/EACMMV and *South African cassava mosaic virus*. Rolling circle amplification, based on next generation sequencing (NGS) data, further suggested the presence of a putative species *Cassava mosaic virus* (MW-KK3S-2013) isolated from the central region. In addition to begomovirus species, Cassava virus C was recovered from samples from Chitipa district using sRNA-derived NGS data.

Molecular analysis of the *P1* gene in CBSV showed a novel and intermediate phylogenetic grouping tentatively called CBSV-Tanzania. Additionally, our Illumina sequencing showed for the first time a genetically divergent UCBSV isolate from Malawi with a geographically distinct monophyletic sub-clade. Analysis of the evolutionary dynamics of the viral gene and the Bayesian coalescent approach using BEAST revealed that the CBSV coat protein was the fastest evolving among studied potyviruses in 2009, 2013 and 2015. Bayesian phylogeography and spatial migration analysis showed molecular

evidence that East Africa is the center of viral diversity. These results are important not only for Malawi but also for the whole cassava-growing region of East and Central Africa as they provide key insights into the evolutionary dynamics of the viruses associated with cassava. Additionally, these results provide a 'wake-up call' for vigilance regarding the possible emergence of novel virus species, strains or subspecies. The search for alternative hosts of CBSVs identified none during the surveys.

The CDP support to seed systems and cassava breeders was achieved through the virus-indexing of 4248 cassava planting materials for CMD and CBSD collected from breeders' plots. Knowledge of cassava stakeholders on cassava virus diseases was enhanced by training 73 extension officers and 52 farmers on various aspects of integrated disease management and on the multiplication of quality planting materials. Similarly, a total of 30,400 virus-tested cassava planting materials were disseminated to farmers through demonstration plots.

The capacity of the country for cassava virus disease-management was further strengthened through the enhanced human resources and physical infrastructure. A total of 13 officers were trained during various short-term professional courses and two scientists pursuing MSc and PhD studies were trained in identifying cassava viruses. In addition, 176 graduate students from various universities were given access to the disease diagnostics laboratory to conduct their research. Similarly, the infrastructure of Malawi in cassava research was enhanced through the upgrading of the water system, the renovation of a screenhouse and the acquisition of various basic and advanced laboratory equipment and consumables necessary for cassava virus research.

Acronyms and abbreviations

5CP	Cassava Varieties and Clean Seed to Combat CBSD and CMD Project
AGRA	Alliance for Green Revolution in Africa
ASWAP	Agriculture Sector-wide Approach project
BEAST	Bayesian Evolutionary Analysis Sampling Trees
CBSD	Cassava brown streak disease
CBSV	*Cassava brown streak virus*
CDP	Cassava Diagnostics Project
CIAT	International Center for Tropical Agriculture
CMB	Cassava mosaic begomovirus
CMD	Cassava mosaic disease
DARS	Department of Agricultural Research Services
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany
EACMCV	*East African cassava mosaic Cameroon virus*
EACMMV	*East African cassava mosaic Malawi virus*
EACMV	*East African cassava mosaic virus*
EPA	Extension planning area
GIZ	Deutsche Gesellschaft für Internationale Zusammenarbeit
IIAM	Instituto de Investigação Agrária de Moçambique
IITA	International Institute of Tropical Agriculture
Illumina	Sequencing technique within NGS technology
JKUAT	Jomo Kenyatta University of Agriculture and Technology
LUANAR	Lilongwe University of Agriculture and Natural Resources
NaCRRI	National Crop Resources Research Institute
NCSU	North Carolina State University
NGS	Next generation sequencing

RAB	Rwanda Agriculture Board
SACMV	*South African cassava mosaic virus*
UCBSV	*Uganda cassava brown streak virus*
TARI	Tanzania Agriculture Research Institute
UWA	University of Western Australia
ZARI	Zambia Agriculture Research Institute

Results summary: Malawi

Aim I: Understand the threat from evolving viruses and vectors

Objective 1: Disease epidemiology

Disease and whitefly prevalence surveys conducted	• Two surveys were carried out in 2013 and 2015 in 24 districts in Malawi. In 2013, a total of 119 fields were surveyed for cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) incidence and severity and adult whitefly populations. A total of 259 leaf samples were collected for analysis in the laboratory, 75 for CMD, 175 for CBSD and nine for wild plants. Only 94 of the 175 CBSD samples were analyzed, as 81 samples had deteriorated. • In the survey performed during 25 June to 10 July 2015, a total of 260 and 192 leaf samples were collected for CMD and CBSD, respectively, from 107 fields across the country. Whitefly samples were collected where present. • Cassava mosaic begomovirus (CMB) sequences were generated as part of Mr Andrew Mtonga's MSc thesis. • *Cassava brown streak viruses* (CBSVs), i.e. *Uganda cassava brown streak virus* (UCBSV) and CBSV, sequences were generated and deposited in NCBI (part of Mr Willard Mbewe's PhD). • Two manuscripts were published (Mbewe et al., 2017a, 2017b).

Objective 2: Characterization of emerging viruses

Cassava virus isolates in the project countries sequenced and analyzed	CMD virus isolates: • Sixteen whole genome sequences were obtained for CMBs in Uganda, Kenya, Malawi, Mozambique, Rwanda, Tanzania and Zambia. CBSD virus isolates: • A divergent UCBSV isolate from Malawi was sequenced, published and deposited in GenBank (Mbewe et al., 2017b). • CBSV isolates were obtained from Tanzania and Malawi. These were sequenced at DSMZ, Germany, and a paper was published (Mbewe et al., 2017a). • A total of 12 full DNA-A CMB sequences from Malawi were generated by MSc student Andrew Mtonga.
Cassava virus distribution maps generated (incidence, severity, whitefly, viruses, sat)	• Disease maps were produced from the survey data by project partner Rothamsted Research and the AgShare.Today team.

Objective 3: Characterization of disease vectors	
Virus population (species) in whiteflies determined and characterized	• Samples from Malawi were checked for viruses using next generation sequencing at the University of Western Australia (UWA) by Dr P. Sseruwagi in collaboration with Dr Laura Boykin in 2014. • These samples used to develop species delimitation.
Aim II: Support clean seed systems for farmers	
Objective 6: Conventional breeding support	
Breeders' material monitored for disease and indexed for virus (CMBs and CBSVs) load at 3, 6, 9 and 12 MAP	• Material from 28 breeders was assessed for CMBs and CBSD. • A total of 4248 samples were indexed for CMBs and CBSVs.
Objective 9: Reaching farmers directly and through partners	
Farmers trained on CMD and CBSD disease symptom recognition and management strategies	• One training event was conducted in Malawi and attended by 25 farmers (12 male and 13 female).
Demonstration plots for benefits of using virus indexed planting materials established on-farm	• 30,000 cassava cuttings were harvested from the cassava multiplication plots at Nathenje and Vinthukutu multiplication fields. • Farmers who participated in this activity (35 male and 17 female) benefited from these planting materials. They were given disease-free cuttings when the cassava roots were harvested.
Information materials developed and disseminated	• The project team participated in the national agricultural fair in Blantyre during 20–30 August 2014. The contrast between clean and diseased cassava was demonstrated. Printed leaflets were disseminated to visitors to the Department of Agricultural Research Services pavilion. • Journalists participated in the Cassava Diagnostics Project (CDP) Fifth Annual Meeting of 11–13 May 2015. • Two articles were published – in print and electronically (*The Nation*, 2015; MBC, 2015).
Aim III: Build sustainable regional capacity	
Objective 10: Strengthening stakeholder linkages	
Awareness on availability of diagnostic capacities created through training and different media	• One media tour was conducted for 12 journalists. • Three articles were published in print media. • A radio program on cassava viral diseases and efforts to combat the problem was aired on the Malawi Broadcasting Cooperation in August 2015.

	• A training workshop was conducted on cassava production, disease recognition and management in Salima district to extension staff in January 2015.
Objective 11: Strengthening human capacity and infrastructure	
Human capacity	
Project staff recruited	• Seven project staff recruited.
PhD and MSc students trained on various aspects of cassava virus diseases	• One PhD student has submitted his thesis, and expects the defense of his thesis to take place by the end of 2018. • One MSc student successfully defended his thesis and was awarded his degree.
Advanced specialized training and visits for project scientists (1–2 months) conducted	• Dr Benesi (Country Team Leader) visited the Agricultural Research Organisation, Volcani Center in Israel during 2–8 February 2014.
Extension workers, crop inspectors and other stakeholders (1 week) training	• Three training sessions were conducted in central, south and northern regions of Malawi. • A total of 73 extension workers were trained (20 female and 53 male).
Project staff trained on IP, biosafety issues and communication strategies	• Dr Benesi and his assistant attended a course on intellectual property rights and communication strategies during 27–31 October 2014 at the World Agroforestry Centre, Nairobi, Kenya.
Project results and information disseminated	Papers published and in preparation: • Two manuscripts published by Mr Willard Mbewe, the project's PhD student (Mbewe et al. 2017a, 2017b) • Two manuscripts are in preparation by Mr. Andrew Mtonga, the MSc student • One MSc thesis by Mr Andrew Mtonga (2018). Molecular characterization of Geminiviruses infecting cassava in Malawi. Makerere University, Uganda. Conference papers presented: • Five conference papers presented by Mr. Willard Mbewe on his PhD research.
Infrastructure strengthening	
Greenhouses constructed/renovated	• Screenhouse renovation completed.
Vehicles, laboratory equipment and consumables procured	• One project vehicle procured and in use • Assorted laboratory consumables, computers and accessories were procured.

Background

In Malawi, cassava is grown on 231,058 ha with an average production output of 8 t/ha, an amount substantially below Africa's average of 10.1 t/ha (FAO, 2016). The crop comes second after maize (Rusike et al., 2009) and supports 30% of the estimated 16 million people. Most of the cassava is grown by small-scale farmers (Rusike et al., 2009). Although cassava is grown all over the country, production is high in the lakeshore areas of Nkhotakota, Nkhatabay, Rumphi and Karonga districts (Figure 1) (Benesi et al., 2004). Cassava forms an important component of the cropping system in Malawi where maize is generally the primary staple crop. Due to its resilience against drought, it is of great value when other crops fail. In addition to being used as a food, cassava is a raw material for industry (Benesi et al., 2004) and feed for livestock.

Figure 1 Map of Malawi showing districts

Cassava in Malawi forms an important part of breakfast and snacks for the majority of Malawians. Its low protein content in roots is complemented by the nutritious green leaves that are rich in protein (17–18%), vitamins and minerals; and are used as a vegetable (Benesi et al., 2004). The leaves are extensively used as relish in Malawian traditional dishes. The leaves are readily available throughout

the year and are particularly useful in the dry season when other green vegetables are in short supply. The crop is increasingly a very important cash crop for smallholder farmers, middlemen and retailers who target fresh markets in both urban and peri-urban areas. Annual production was estimated at 5 million tonnes in 2015 (Figure 2).

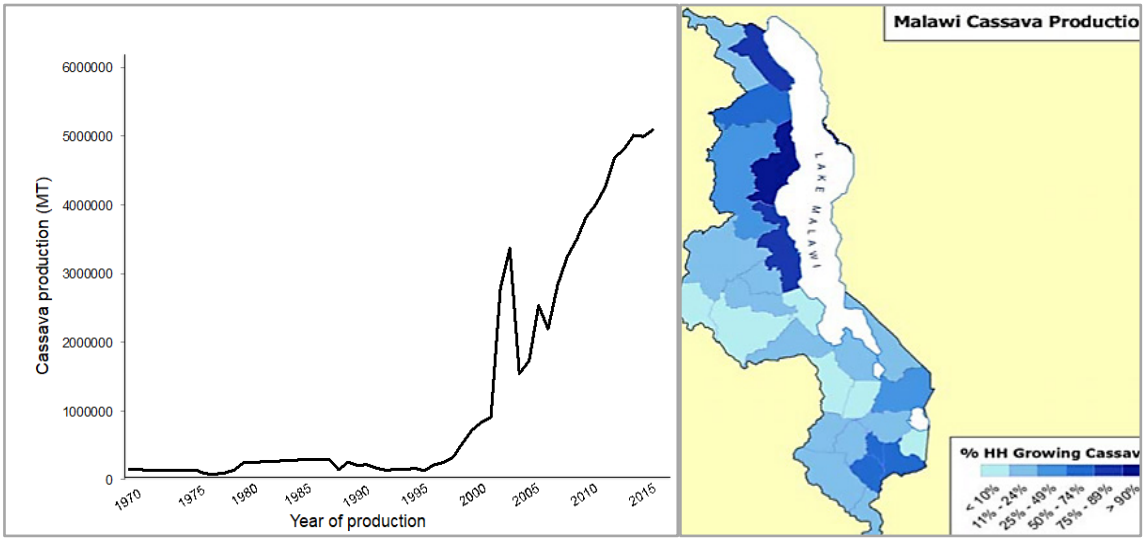

Figure 2 (left) Cassava production trends: 1970–2015. Source: FAOSTAT, 2016 and (right) cassava cultivation areas in Malawi

The trend in cassava production indicates that the crop will become even more important in future years (Figure 2). This is because, in recent years, commodity prices have favored cassava over maize as a result of removal of consumer and producer subsidies. Other factors contributing to maize decline are the development, dissemination and adoption of improved technologies; the collapse of input supply, credit and maize markets; declining soil fertility below economic yields for maize; and high rainfall variability. All these factors have contributed to the ever-increasing production of cassava (Haggblade et al., 2012).

As in many parts of Africa, cassava production in Malawi suffers from a range of biotic and abiotic constraints. Examples of these include unpredictable or unfavorable weather patterns and a number of pests and diseases that result in low productivity. Pests such as whitefly (*Bemisia tabaci* Gennadius), cassava green mite (*Mononychellus tanajoa* Bonder) and cassava mealybug (*Phenaccocus manihoti* Matile-Ferrero) cause serious mechanical damage to the crop by directly feeding on different plant parts (Calvert & Thresh, 2002; Campo et al., 2011; Legg et al., 2015). In addition to causing direct physical damage, *B. tabaci* is a known vector of cassava viruses (Maruthi et al., 2005).

The current major threats to the cassava health and productivity are (1) cassava mosaic disease (CMD) caused by CMBs (family *Geminiviridae*; genus *Begomovirus*); and (2) cassava brown streak disease (CBSD) caused by *Cassava brown streak virus* (CBSV) and (3) *Ugandan cassava brown streak virus* (UCBSV) (both of family: *Potyviridae*; genus *Ipomovirus*) (Mbanzibwa et al., 2009; Winter et al., 2010; King et al., 2012). Unlike other crops that have a short season, cassava has a long growth cycle ranging within 6–24 months. This long growth cycle increases the crop's exposure to viruses and their vectors. Furthermore, since cassava is a vegetatively propagated plant, virus spread is to a large extent through cuttings (Legg et al., 2015).

SECTION ONE: Understanding the threat from evolving viruses and vectors

Disease epidemiology

Three country-wide cassava virus disease monitoring surveys were carried out in 2009, 2013 and 2015. The objective of the study was to determine the status of CMD and CBSD and to characterize the associated viruses and/or strains. Sampling was carried out in accordance with the harmonized protocol adopted by all Cassava Diagnostics Project (CDP) country partners (Sseruwagi et al., 2004).

In the 2013 country-wide survey, conducted from 20 July to 6 August 2013, a total of 119 fields were surveyed for CMD and CBSD incidence and severity and adult whitefly populations. A total of 259 leaf samples were collected for analysis in the laboratory: 75 for CMD, 175 for CBSD evaluation and nine for uncultivated plants. Only 94 of the 175 CBSD samples collected were analyzed, 81 samples having deteriorated.

In the 2015 survey, carried out from 25 June to 10 July 2015, a total of 260 and 192 leaf samples were collected for CMD and CBSD, respectively, from 107 fields across the country. Whitefly samples were collected where present.

CMD and CBSD incidence

Incidence is the proportion of plants that show symptoms of disease, and severity is the average of diseased plants assessed during the survey.

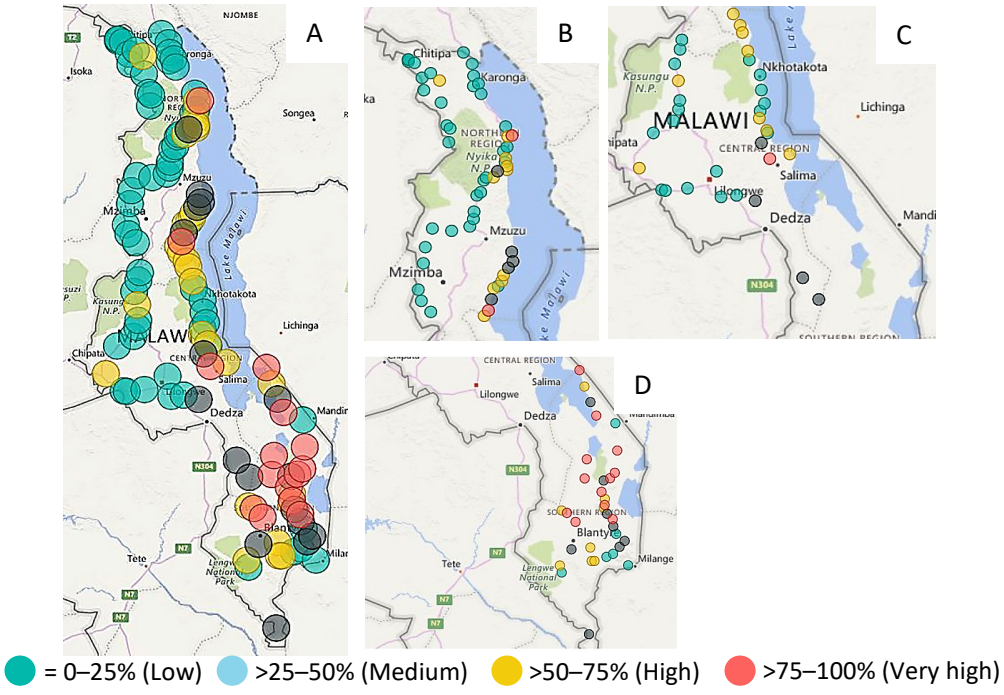

Figure 3 CMD incidence 2013 survey: (A) Overview of incidence at locations surveyed. Detailed incidence levels in Northern Malawi (B) Central Malawi (C) and Southern Malawi (D)

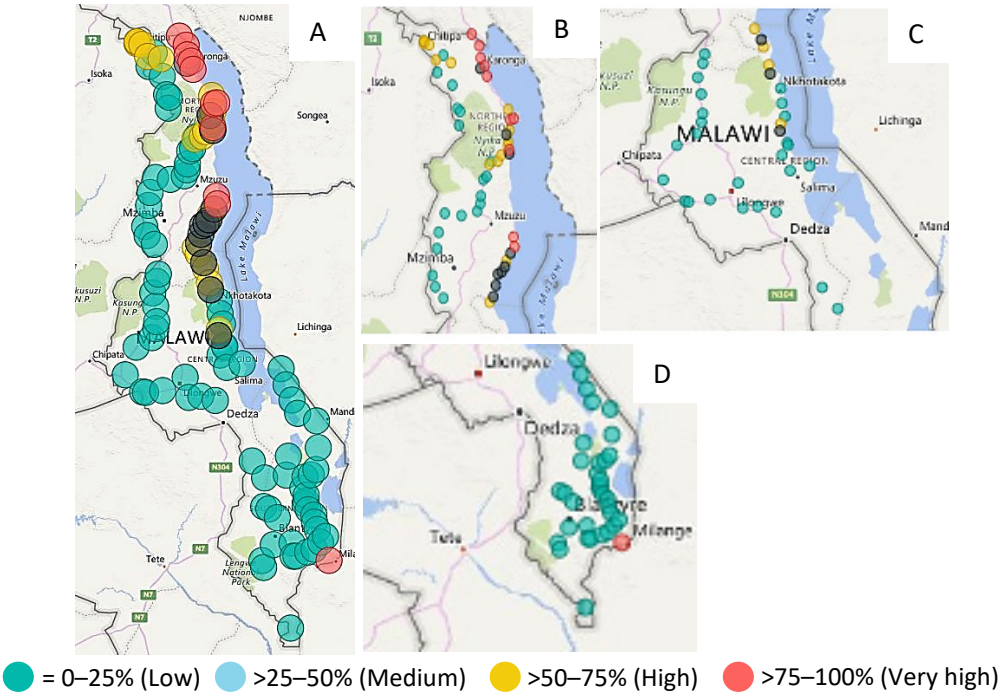

Figure 4 CBSD incidence 2013 survey: (A) Overview of incidence at locations surveyed. Detailed incidence levels in Northern Malawi (B) Central Malawi (C) and Southern Malawi (D)

In 2013, CMD was observed throughout all districts and in almost all fields sampled except for Mzimba district, but incidence varied considerably among those districts and regions (Figure 4 and Figure 5). Survey data showed that CMD incidence was generally low in the northern (19.2%) and central region (21.9%) and highest in the southern region (57.8%). Using the severity index range of 0–5 (where 5 indicates a high level of infection), mean CMD severity ranged within 2.2–2.8. Individual district scores showed that Nsanje (Southern region) had the highest score (3.9).

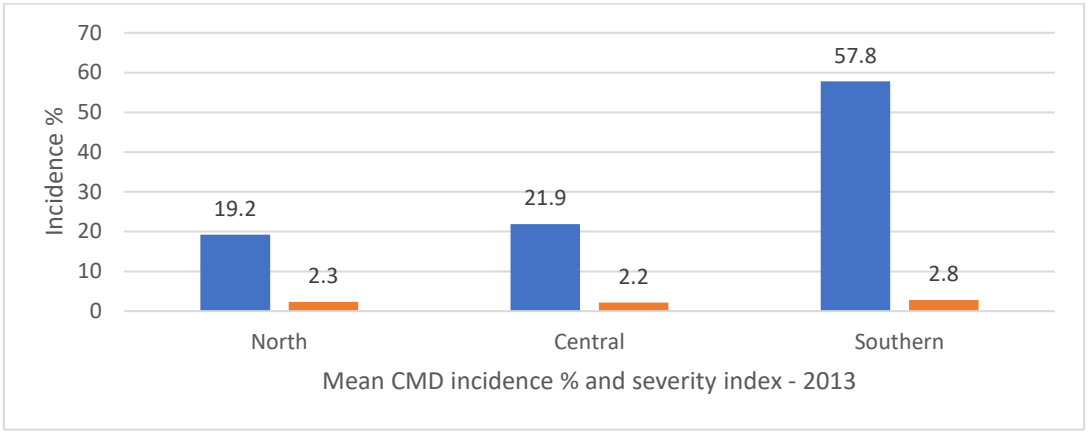

Figure 5 CMD incidence % and severity index – 2013 survey

Similarly, in the 2015 survey, CMD incidence was lowest in northern region and highest in the south (Figure 5 and Figure 7). The CMD incidence varied considerably among the districts surveyed. At individual district level, it was highest in Balaka in the south (100.0%) and lowest in Chitipa in the north (2.2%). This pattern, as also seen in 2013, suggests that Balaka could be one hotspot area for CMD. Individual district scores showed that 33% of districts in the south had a severity score exceeding 3.

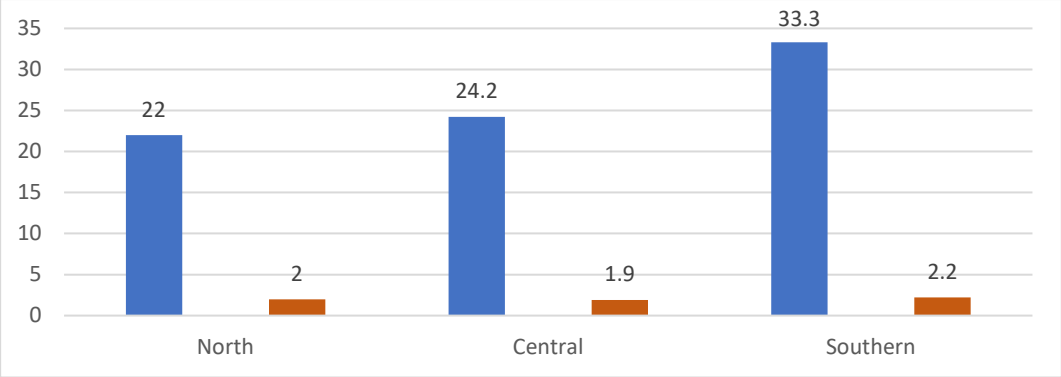

Figure 6 Mean CMD incidence % and severity index – 2015 survey

CBSD incidence and severity

The CBSD foliar incidence is the percentage of plants expressing CBSD symptoms in leaves. In the 2013 survey (Figure 7), there was high variation in CBSD incidence among the districts sampled, in the range of 0–79.3%. Highest CBSD incidence was in Karonga (79.3%), a district bordering Tanzania. The CBSD incidence was high in the northern (38%) and lowest in the southern region (5.9%). The average CBSD foliar severity was moderate in all districts surveyed, with a mean score of 2.4.

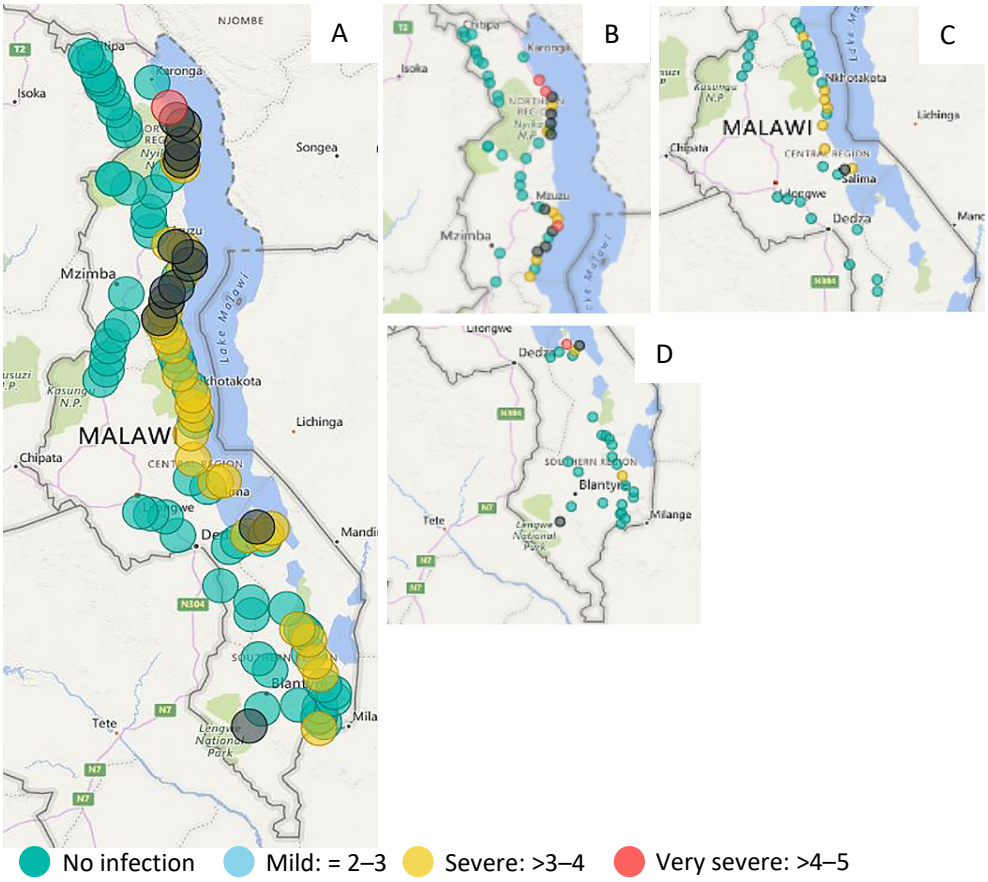

Figure 7 CBSD incidence 2015 survey: (A) Overview of incidence at locations surveyed. Detailed incidence levels in Northern Malawi (B) Central Malawi (C) and Southern Malawi (D)

In 2015, CBSD incidence varied among the districts surveyed, ranging within 1.3–70.7%. On average, the northern region had the highest (25.1%) CBSD incidence compared to the south (11.0%) and central (11.6%) regions. Similarly, the 2013 survey showed high CBSD occurrence in the northern region compared to the other two regions. Thus, the CBSD distribution had not changed much.

However, the CBSD incidence in the southern region had increased. Among the 21 districts sampled, CBSD incidence was highest in Salima district (70.7%) and lowest in Mulanje district (1.3%). The CBSD severity score varied considerably among the districts sampled: highest in Karonga (3.4) and lowest in Machinga (2.0) district.

Whitefly abundance

The mean number of adult whiteflies per plant was generally low in all districts surveyed in 2013, with an overall average of 0.7 whiteflies per plant. The highest number of adult whiteflies per plant was in Phalombe districts (5.1), followed by Chikwawa (3.2) and Mulanje (3.9) districts (Table 1). The low numbers of adult whitefly suggest that the CMD and CBSD incidence observed during the study were mainly due to use of infected planting materials and not to virus activity.

Table 1 Mean whitefly abundance in districts in the three regions surveyed in 2013 and 2015

Region	Fields	No. adult whiteflies/plant	Region	Fields	No. adult whiteflies/plant
	2013			**2015**	
North			**North**		
Chitipa	12	0.2	Chitipa	12	0
Karonga	10	1.9	Karonga	6	0.4
Rumphi	10	0.8	Rumphi	7	0.8
Nkhatabay	10	0.6	Nkhatabay	12	0.5
Mzimba	11	0.1	Mzimba	6	0
Central			**Central**		
Dedza	1	1.3	Dedza	2	0
Ntcheu	2	0.6	Ntcheu	3	1
Kasungu	8	0	Kasungu	10	0.3
Mchinji	3	1.3	Mchinji	1	0.2
Lilongwe	4	0.2	Lilongwe	3	0.2
Salima	4	0.3	Salima	6	1
Nkhotakota	10	0	Nkhotakota	13	0.4
Southern			**Southern**		
Blantyre	3	0.2	Blantyre	3	0
Chikwawa	2	3.2	Chikwawa	1	2.5
Chiradzulu	1	0	Chiradzulu	1	0
Mulanje	3	3.9	Mulanje	5	0.3
Phalombe	3	5.1	Phalombe	3	2.2
Zomba	6	0.3	Zomba	5	2.5
Machinga	5	0	Machinga	2	0
Mangochi	5	0.1	Mangochi	6	1.9
Balaka	2	0.2	Balaka	1	0

Similarly, in 2015, whitefly mean counts were very low in all districts surveyed, ranging from 0.3 to 2.5 adult whiteflies per plant, and an overall national mean of 0.6. The highest whitefly count was observed in Southern region.

Characterization of emerging viruses

Cassava virus isolates sequenced and analyzed

Plant materials from field surveys across Malawi were subjected to molecular tests using CMB and CBSV specific primers and were consequently sequenced using both the Sanger method and Illumina deep sequencing techniques.

A total of 26 sequences were generated for phylogenetic and evolutionary analyses using (BEAST) the Bayesian Evolutionary Analysis Sampling Trees software (Suchard et al, 2018) – see Figure 8. In addition to cassava viruses previously identified in Malawi – CBSV, UCBSV, *East African cassava mosaic Malawi virus* (EACMMV), *East African Cassava Mosaic Cameroon virus* (EACMCV) and *South African cassava mosaic virus* (SACMV) – our analyses revealed the presence of previously unreported Cassava virus C (Mtonga, 2018) and an Ampelovirus (Mbewe, unpublished) from Malawi. The data were not adequate enough to suggest that Cassava virus C isolate from Malawi was a species distinct from the Ivory Coast isolate. A separate finding was that computational analysis resulted in identifying a divergent CBSV subgrouping based on the *P1* gene (Mbewe et al., 2017a), as well as a genetically divergent UCBSV isolate (Mbewe et al., 2017b).

Cassava mosaic begomoviruses

Several begomovirus species and strains causing CMD have been reported in Malawi. In the 2013 survey, cassava leaf samples with conspicuous CMD symptoms were collected and subjected to PCR. A total of 259 DNA samples were analyzed for CMBs, of which 111 (43%) tested positive. EACMMV was detected in northern, central and southern regions of Malawi, while SACMV was only detected in southern region. No EACMCV was detected in the 259 samples analyzed. A total of 16 (6.4%) samples tested positive to dual infections. The common dual infection was EACMMV + SACMV (15), and only one sample tested positive to EACMMV + EACMCV and none to EACMCV + SACMV. Of all districts surveyed, Nkhatabay had the highest occurrence of EACMMV (eight), followed by Mzimba (seven). Fourteen samples were positive to universal EAB555F/EAB555R (Ndunguru et al., 2005) but did not amplify any of the specific primers. It is possible that these belonged to *East Africa cassava mosaic Zanzibar virus* and *East Africa cassava mosaic Kenya virus*.

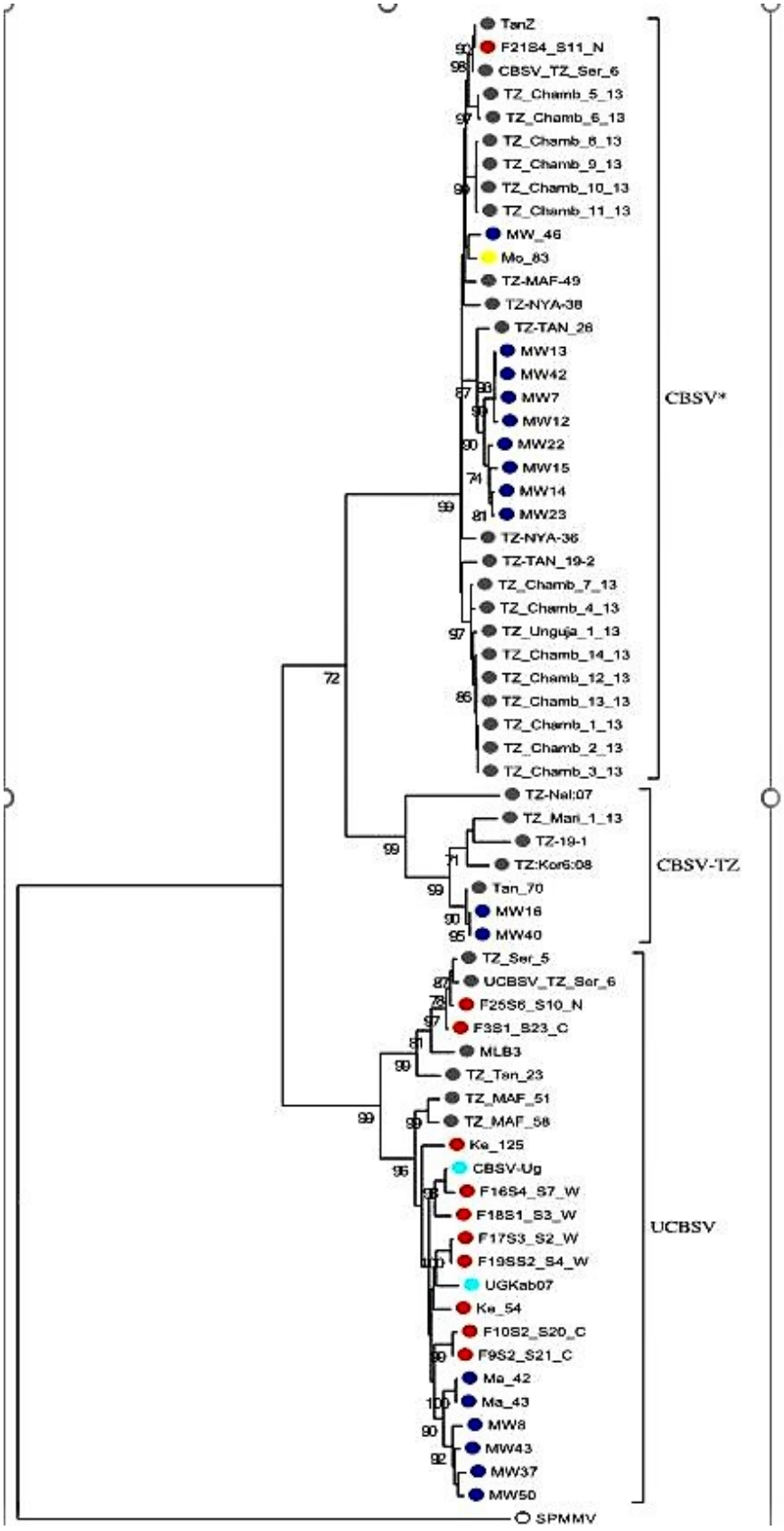

Figure 8 Maximum likelihood tree of partial *P1* gene sequences of CBSV isolates (adapted from Mbewe et al., 2017a)

In the 2015 survey, out of 257 samples analyzed using PCR, 65 (25.3%) were positive for cassava mosaic begomoviruses (CMB). The EACMMV was the most common CMB in Malawi with 45 (69.23%) samples positive for EACMMV, and only one sample tested positive to EACMCV (Figure 9). There was co-infection of EACMMV + EACMCV in 15 samples representing (23.1 %), and EACMMV + SACMV in one sample (0.4%). Three samples (4.61%) had all three viruses: EACMMV + EACMCV + SACMV.

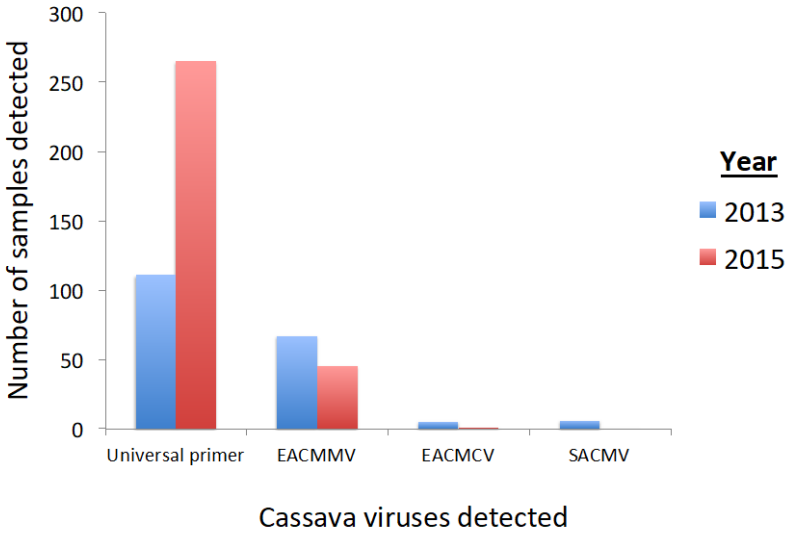

Figure 9 Detection of cassava mosaic begomoviruses in Malawi

Ninety-four RNA samples were analyzed, of which 29 (31.9%) gave positive results. Among these, 11 (37.93%) had single infection of CBSV, 15 (51.72%) had UCBSV alone and three (10.34%) had dual infection of CBSV + UCBSV. Single UCBSV infection was prevalent in Nkhatabay (10), while single CBSV was most frequent in Rumphi (five) and Karonga (four). The CBSV results suggest that UCBSV is the most common species in Malawi.

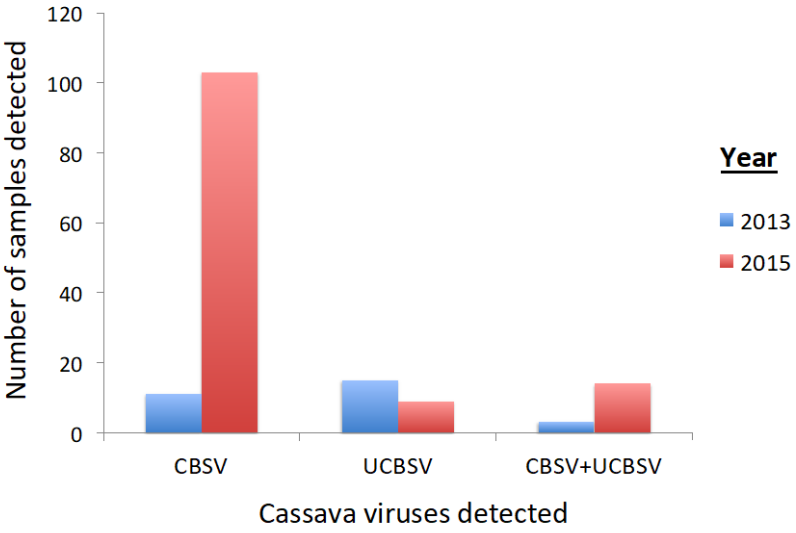

Figure 10 Detection of cassava brown streak ipomoviruses in Malawi

In the 2015 survey, a total of 192 cassava leaf samples were analyzed for CBSVs, of which 58.3% tested positive to CBSV (103) and UCBSV (nine), suggesting that CBSV was the most common species in Malawi. This differed from the 2013 survey, in which UCBSV was common (Figure 10). CBSV occurred in all three regions of Malawi, while UCBSV was detected in the northern and central regions but not in the southern region. Prevalence of CBSV was high in Nkhotakota (18) and Nkhatabay (16), and UCBSV was high in Nkhatabay (4) district. A total of 14 samples (12.61%) had dual infection of CBSV + UCBSV. All samples collected from Chitipa and Mzimba were free of CBSVs.

Changes in cassava virus disease incidence 2013–2015 surveys

- Mzimba was CMD-free in 2013 but CMD was present in 2015
- There was no SACMV in the northern region in 2013 but it was detected in 2015
- Balaka had the highest CMD incidence in both surveys
- Southern region had the highest CMD incidence in both surveys
- Northern region had the highest CBSD occurrence.

In 2013, UCBSV was high; however, in 2015, CBSV were much more common than UCBSV. CBSD was distributed more along the lakeshore, but laboratory results showed higher CBSV incidence in the south than in previous surveys. This calls for strategies to slow or stop the spread of CBSD in the country. The CMD incidence was high along the lakeshore and in the southern region compared to the upland areas of central and northern region. These areas of low incidence for both CMD and CBSD would be suitable for establishment of cassava planting materials.

Alternative hosts for CBSVs and CMBs and associated insect vectors identified

Survey of neighboring uncultivated plants

During the survey, leaf samples of wild plants with virus-like symptoms were collected for virus testing in the laboratory with the aim of identifying the potential reservoir host for viruses in Malawi. Seven plant species showing CBSD-like (brown streaks) and CMD-like (green or yellow mosaic) symptoms were collected during the survey for virus testing (Table 2). These plants were tested for presence of CBSVs and CMBs and all were found to be free from both viruses and were not found of immediate concern to the cultivation of cassava in the areas surveyed. All samples tested negative for both CMD and CBSD in the laboratory analysis.

Although all the wild samples tested negative, this does not mean they were clean of virus and this can be caused by the specificity of primers used. Deep sequencing techniques could reveal possible viral presence and establish evolutionary relationships between possible novel viruses and those from cassava fields. This is an area to be considered in the future.

Table 2 Symptomatic uncultivated plants tested for CMD and CBSD

Uncultivated plants	District	Symptoms	Result of test
Pumpkin (*Curcubita* spp.)	Balaka	CMD-like	Negative
Fig tree (*Ficus carica*)	Nkhatabay	CBSD-like	Negative
Avocado (*Persea americana*)	Nkhatabay	CBSD-like	Negative
Pawpaw (*Carica papaya*)	Mangochi	CMD-like	Negative
Lemon tree (*Citrus limon*)	Mangochi	CBSD-like	Negative
Pawpaw (*Carica papaya*)	Rumphi	CMD/CBSD-like	Negative
West Indian lantana (*Lantana camara*)	Nkhatabay	CMD-like	Negative

Characterization of disease vectors

Whitefly characterization

To identify potential insect vectors of cassava viral diseases, whitefly and other insect samples were collected during the surveys. A total of 67 whitefly samples were collected for characterization during the 2013 and 2015 surveys. Project staff were trained on whitefly DNA extraction and on the detection of viruses from the extracted DNA. Dr Sseruwagi and Ms Leonia Mlaki from the Tanzania Agricultural Research Institute (TARI)–Mikocheni, Tanzania, facilitated the training. Preliminary results indicated the occurrence of *B. tabaci* SSA1 species with two genetic groups: Subgroup 2 and Subgroup 3.

SECTION TWO: Integrated pest management

Supporting clean seed systems for farmers

Conventional breeding support

Breeders' material monitored for disease and indexed for virus (CMBs and CBSVs)

The human capacity on cassava viral diagnostics that was developed under the CDP has benefited other projects that were working toward the availability of virus-free planting materials for cassava.

Breeders' seed and multiplication plots were monitored for CMD and CBSD, and leaf samples were collected for virus indexing. A total of 4248 cassava leaf samples were collected and screened for cassava viruses (Table 3) from regional multiplication trials under the Cassava Varieties and Clean Seed to Combat CBSD and CMD Project (5CP) project from five sites in Malawi: Makoka, Chitedze, Chitala, Mkondezi and Vinthukutu.

Table 3 Samples indexed for other projects

Project	Disease screened	Number of samples analyzed	Virus free	Virus infected
ASWAP-SP initiative[1]	CMD & CBSD	1500	1066	434
IITA[2]-GIZ[3] funded project on cassava commercialization[2]	CMD & CBSD	1500	1454	46
AGRA-funded project on breeder and foundation seed multiplication[4]	CMD & CBSD	1248	711	537
Total		**4248**	**3231**	**1017**

[1] Agriculture Sector-wide approach support program (ASWAP-SP); [2] International Institute of Tropical Agriculture (IITA), [3] Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ); [4]Alliance for Green Revolution in Africa (AGRA)

Reaching farmers directly and through partners

Training farmers on CMD and CBSD disease symptom recognition and management strategies

Baseline survey on farmers' knowledge on cassava viral diseases

The objective of the survey was to obtain information on farmers' knowledge on cassava viral diseases, farmer cultivation practices and how they influence the spread of viral diseases (Figure 11). Farmers were interviewed on their years of experience in cassava cultivation, constraints in cassava cultivation, preferred varieties and access to extension services.

Farmers interviewed mentioned Sauti, 20:20, Kolobeka, Matako Lembwende and Nyantonga as their preferred varieties due to being early maturing, high yielding and disease resistant. These surveys indicated that there was a need for improved, disease-free cassava planting materials and training in better farming methods and management of cassava virus diseases.

Figure 11 Project staff doing national disease survey in 2015

In 2016, farmers who participated in the demonstration plots were trained on the identification and management of cassava pests and diseases, clean cassava seed production and good agronomic practices for cassava cultivation. The training was conducted during 2–5 October 2016 when the plants were eight months old, and was attended by 25 farmers (12 males and 13 females) and three male extension staff.

Training of extension workers on cassava disease identification and management

In line with this objective, two-day training events were organized in 2015 in each of the three regions of Malawi. Two training sessions were organized jointly with the project coordinator for the ASWAP project. A total of 73 participants including extension staff and research technicians were trained (Table 4).

Table 4 Summary of training for extension staff and research technicians

Region	Females	Males	Total
North	4	22	26
Central	11	23	34
South	5	8	13
Total	**20**	**53**	**73**

To ensure that all participants shared a common understanding of the information disseminated, the training focused on harmonizing messages on viral disease identification, and on the management and implementation of trials.

Cultivating demonstration plots to underline the benefits of using virus-indexed plant material

The objective was to demonstrate the benefits of using improved and indexed cassava planting materials to farmers. The demonstrations were designed so that the demonstration plots would be hosted by a group of farmers, and that the planting would comprise two cassava varieties: an improved variety identified through research and one farmer-preferred local variety. However, in the first year, it was not possible to organize farmers into groups, and thus demonstrations were hosted by lead farmers. Farmers from the surrounding communities were invited on specific occasions for training on CMD and CBSD symptom recognition and management strategies.

Three demonstration plots were set up in each of the 2014/15 and 2015/16 seasons: two in Vinthukutu and one at Nathenje using indexed planting materials along with one local variety. At both sites the indexed varieties used were Sauti, Sagonja, Kalawe and Mpale. The following local varieties were used: Mbundumali at Nathenje; and Matakolembwende and Nyautonga at Vinthukutu. In December 2015, the demonstration field at Nathenje was ratooned and planting materials were distributed to farmers for further multiplication. The plot was maintained, and materials harvested in December 2016, and planting materials were shared to 25 farmers within the Nathenje area.

The 2014/15 demonstration plots in Karonga were not harvested as they were damaged by livestock. In the 2015/16 season, two demonstration plots were established in the village of Mng'ombwa in the Hara section of Vinthukutu Extension Planning Area (EPA). Farmers were given virus-free planting material from varieties Sagonja, Sauti, Mpale, Kalawe and Mbundumali to plant in the demonstration plots along with their chosen local variety. Farmers near the demonstration plots were trained in cassava disease and pest identification and management.

During the 2016/17 season, two new farmer groups were formed and given clean planting materials. Each demonstration group (Mwawi and Kambwera at Vinthukutu EPA in Karonga district) had a plot of 10 ridges by 10 m where one improved variety (Sagonja) and a local variety (Matakolembwende) were planted. At each demonstration plot, a bulk crop of Sagonja variety (approximately one acre) was multiplied.

Planting materials from the demonstrations and seed multiplication were distributed to farmers. A total of 52 farmers benefited from the clean planting materials harvested from plots at Vinthukutu and Nathenje (Table 5).

Table 5 Cuttings distribution and farmer demographics

Region	Number of cuttings shared	Female	Male	Total
Northern	9800	2	5	7
Central	20,600	15	30	45
Total	**30,400**	**17**	**35**	**52**

Development and dissemination of information materials

Disease awareness

To facilitate message dissemination on cassava viral diseases, a media tour was organized during 21–23 June 2015 – a total of 12 journalists from seven media houses participated. The tour started with formal training on causes, symptoms and management of both CMD and CBSD, followed by a field visit to cassava fields for a practical session on disease recognition (Figure 12).

Figure 12 Journalists training on cassava viral diseases, Salima, June 2015: (left) participants during formal training in class, (right) participants during practical field session

Apart from the media tour, journalists also participated in the CDP Fifth Annual Meeting held at the Golden Peacock Hotel, Malawi, during 11–13 May 2015. The Minister of Agriculture Dr A. Chiyembekeza officially opened the meeting. After this meeting two articles were published – in print and electronically (MBC, 2015; The Nation, 2015).

Participation in agricultural shows and exhibitions

The project team participated in one national agricultural fair held in Blantyre during 28–30 August 2014. During the fair, the team displayed samples of clean and diseased cassava planting materials, posters on the incidence and severity of cassava virus diseases in Malawi in 2013 and different cassava varieties. The President of the Republic of Malawi and Chief Justice were the high-profile guests at the Department of Agricultural Research Services (DARS) pavilion (Figure 13).

Figure 13 National Agricultural Fair at Trade Fair Grounds, Blantyre, August 2014. (A) Department of Agricultural Research Services pavilion, (B) print materials on display, (C & D) Director of Research briefing the President of The Republic of Malawi, HE Prof Arthur Peter Mutharika

Field days participation

The team participated in two field days. On 25 April 2016, the team participated in a field day organized by Lilongwe Agricultural Development Division. The field day took place at Nathenje EPA where a demonstration had been established under this project (Figure 14). The carry-home message was use of improved varieties and clean planting materials. The field day was attended by 178 farmers: 70 male, 86 female and 22 children.

Figure 14 Farmers participating in the Nathenje Field Day 2016

On 31 March 2017, the team participated in field day organized by Chitedze Research Station (Figure 15). The message centered on use of clean planting materials, and CMD and CBSD recognition and management. Some of the guests who visited our pavilion were farmers, students,

chiefs and community leaders. The guest of honor was the Deputy Minister of Agriculture, Mr. Aggrey Massi, accompanied by some members of the Parliamentary Committee on Agriculture.

Figure 15 Farmers participating in the Nathenje Field Day 2016

Official visitors

Four members of parliament from the Republic of Ireland visited Malawi in July 2015 (Figure 16). They visited the Chitedze Biotechnology Laboratory where they were briefed by the Country Team Leader on activities being carried out and the future plans for the laboratory. The purpose of the visit was to get an appreciation of what a sister project (funded by Irish Aid) was doing to address food security in Malawi. This project made use of the CDP-funded biotechnology laboratory.

Figure 16 Members of Parliament from Ireland visiting Chitedze Biotechnology Laboratory, July 2015

Building sustainable regional capacity

Strengthening stakeholder linkages

Stakeholders engagement

Meetings were conducted annually with the CDP team from Tanzania Agriculture Research Institute (TARI)–Mikocheni. Discussions during these visits enabled the Malawi team to carry out their activities and meet their objectives.

Table 6 Partners and stakeholders visited during the impact assessment baseline study and monitoring and evaluation missions for the 'Disease diagnostics for sustainable cassava productivity in Africa' project, 2014

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
Jomo Kenyatta University of Agriculture and Technology (JKUAT)	Thika Road, Nairobi	University (Project partner)	Dr Elijah Ateka (Project Country Team Leader), Samuel Mwaura (Research Assistant), Timothy Makori (Technician)	Project management, training, supervision of students and research	Dr Elijah Ateka and Mr. Samuel Mwaura
Department of Agricultural Research Services, Chitedze Agricultural Research Station	Lilongwe	NARS (Project Partners)	Dr Ibrahim Benesi (Team Leader), Mr Albert Mhone (Assistant Team Leader), Ms Ruth Semu Mwase (Research Assistant), Mr Michael Benjala (Research Assistant)	Hosting the project, breeding for resistance to CMD and CBSD and disease diagnostics	Dr Ibrahim Benesi, Mr Albert Mhone, Ms Ruth Mwase, Mr Michael Benjala
Department of Agricultural Research Services, Chitedze Agricultural Research Station	Lilongwe	NARS (Non-Project Staff)	Mr Elisa Mazuma (Plant Pathologist and Deputy Director Plant Protection), Mr David Kamangira (Senior Deputy Director of Technology Management and Reg Services), Ms Clementina Banda (Ass Agric Research Officer), Mr Harry Mleta (Agric Research Officer), Mr John Mphaya (Agric Research Officer)	Research and Improvement of cassava	Mr Elisa Mazuma, Mr David Kamangira

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
Department of Agricultural Research Services, Chitedze Agricultural Research Station	Lilongwe	NARS	Ms Sarah Chilungo (Breeder), Mr Wilson Chifudsa (Head of Seed Quality Control Unit)	Cassava breeding for improvement and resistance to CMD and CBSD and Seed Certification	Ms Sarah Chilungo, Mr Wilson Chifudsa
University of Malawi	Lilongwe	Collaborator	Dr Alfred Maluwa (Chairman of National Working Group of Sweet Potato Innovation Platform)	Research and Improvement of cassava	Dr Alfred Maluwa
Lilongwe University of Agriculture and Natural Resources, Department of Crop and Soil Sciences	Lilongwe	University	Dr Moses Maliro (Lecturer in Plant Breeding)	Training and Supervision of Students	Dr Moses Maliro
International Institute of Tropical Agriculture (IITA), Malawi	Lilongwe	BMGF supported project on cassava breeding - New Cassava Varieties and Clean Seed to Combat CBSD and CMD (5CP)	Mr Hastings Musopole	Cassava Breeding and Improvement	Mr Hastings Musopole
International Institute of Tropical Agriculture (IITA), Malawi	Lilongwe	Collaborator	Mr Christopher Moyo (Research Associate – Cassava Breeding) Regional Cassava Breeding Project	Cassava Breeding and Improvement	Mr Christopher Moyo
International Potato Centre (CIP)	Lilongwe	Collaborator/ Private Seed Producer	Dr Philip Demo (Country Representative)	Cassava Breeding and Seed multiplication and distribution	Dr Philip Demo
Ministry of Agriculture and Food Security, Department of Extension Services	Lilongwe	Government extension	Dr Clodina Chowa (Deputy Director Extension Methodology and systems)	Training of farmers and cassava seed multiplication and distribution	Dr Clodina Chowa
Ministry of Agriculture and Food Security, Department of Extension Services	Chilumba Extension Planning Area, Karonga	Government extension	Mr Dingani Zalilo (Extensionist), Mr Jeremiah Moyo (Extensionist)	Training of farmers and cassava seed multiplication and distribution	Mr Dingani Zalilo

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
Farmers	Chambogho, Karonga	Farmer	Ms Teleza Zgambo, Ms Febbie Mzembe, Mr Elijah Mwafulirwa, Mr Wachepa Mwafulirwa and Mr Vasco Msiska	Cassava production	Mr Elijah Mwafulirwa

Collaborations

The facilities and expertise acquired as a result of CDP enabled the Malawi team to work with external organizations and educational institutions (Table 7).

Table 7 Collaboration with external organizations

Institution	Nature of collaboration	Contact person
Bvumbwe Research Station	Genotyping of breeders' seed for regional trial (5CP)	Dr Obed Mwenye
Lilongwe University of Agriculture and Natural Resources (LUANAR)	• BSc Students carrying out their practicals on CMD and CBSD • MSc student carrying out their research using the CDP facility	Dr Abel Sefasi Misheck Bushiri
International Institute of Tropical Agriculture (IITA)	CMD and CBSD diagnostics for IITA-GIZ cassava multiplication fields	Dr Pheneas Ntawarunga, Chris Moyo
Agricultural extension Services (Karonga ADD-Vinthukutu EPA)	Cassava seed multiplication and demonstrations Training of trainers	Extension officer, Vinthukutu EPA Extension Officer, Nathenje Training Centre
University of Free State, South Africa	Masters student, registered with University of the Free State, used CDP facilities toward his Master's thesis	Hastings Musopole

Strengthening human capacity and infrastructure

The CDP has greatly enhanced Malawi's human resource capacity (Table 8). Training undertaken by project personnel includes the molecular detection of cassava viruses, financial management and bioinformatics. A total of 13 officers have been trained. Additionally, the CDP project funded one MSc and one PhD student at Rutgers University, USA, and Makerere University, Uganda.

Table 8 Training courses undertaken by project staff during Phase II of CDP

Dates	Course title	Venue	Staff
May 2013	Financial Management and Accounting package	Dar es Salaam, Tanzania	Dr Benesi, Neston Chimosola and Joel Kwelepeta
22 September to 5 October 2013	Molecular detection of plant viruses	Dar es Salaam	Albert Mhone and David Mbalangwe
July 2014	Leadership Training for institutional Directors	Kigali	Dr Makumba
August 2014	Molecular detection of plant viruses	Dar es Salaam	Sarah Chilungo and Michael Benjala
26–31 October 2014	Intellectual Property Rights	Nairobi, Kenya	Albert Mhone
2014	PhD Research work	DSMZ Plant Virus Department, Germany	Willard Mbewe
2014–2017	MSc	Makerere University	Andrew Mtonga
22–24 February 2016	Bioinformatics	Dar es Salaam	Andrew Mtonga and Albert Mhone
11–13 April 2016	Financial Management Essentials	Dar es Salaam	Albert Mhone and Lucy Chapweteka
June 2016	AgShare Training	Lusaka, Zambia	Willard Mbewe and Andrew Mtonga
December 2016	Data Entry and Assembly Workshop	Dar es Salaam	Ruth Semu and Ruth Kaunda
19–22 January 2017	Leadership Training for Scientists	San Diego, California, USA	Willard Mbewe and Andrew Mtonga
March–April 2017	Analysis of siRNA sequencing data for characterization and discovery of viruses	CIAT, Colombia	Andrew Mtonga

Apart from providing the project staff and government workers with the tools and environment required for research, the CDP project support to the Chitedze Biotechnology Laboratory has benefited several stakeholders, especially students from colleges and universities. During the project period, LUANAR has sent students (Diploma, BSc and MSc students) for familiarization with equipment and procedures used in a biotechnology laboratory. A total of 176 students had access to the laboratory (Table 9). In addition to these short visits, biotech students from LUANAR have been attached to the laboratory as part of their course requirement. During the students' attachment, detection of viral diseases in cassava was one of the major practical activities they undertook.

Table 9 Student visitors to the Chitedze Biotechnology Laboratory

Student category	Areas of specialization	Numbers
Masters	Horticulture, plant breeding, virology	13
Bachelors	Crop sciences, agriculture, biotechnology	143
Diploma	Agriculture	20
Total		**176**

Infrastructure strengthening

The DARS benefited from several infrastructural support items in Phase II of the CDP. These include screenhouse renovation, project vehicle and laboratory equipment.

Access to water

The laboratory has been operational since 2014 but without running water. Water for washing glassware and for other laboratory uses was collected from other offices and hand delivered. In 2017, a 5000-L tank was erected and connected to the laboratory courtesy of CDP.

Renovation of screenhouse

One screenhouse was renovated in 2013 and early 2014 under this project to complement efforts from the disease diagnostics laboratory for the provision of clean cassava planting materials. Cassava plants that have been tested as virus-free from the laboratory were planted in tubes and kept in the screenhouse to protect them from virus disease infection caused by whiteflies.

Laboratory equipment

A number of laboratory equipment items were purchased within the project period. These include gel documentation system, double water distiller, analytical balance, micropipettes, deep freezer and upright fridge (Table 10). The project also supported the procurement of laboratory consumables.

Table 10 Equipment purchased by DARS 2013–2017

Asset	Serial no.	Date of purchase	Current location	Status
Deep freezer	KCG 570/7	2017	Chitedze Biotech Lab	Working order
Upright fridge	BHSTDJ 1642582	2017	Chitedze Biotech Lab	Working order
Water distiller	FI-0020034	2015	Chitedze Biotech Lab	Working order
Gel documentation system	A 113554	2015	Chitedze Biotech Lab	Working order
Analytical balance	ME 204	2015	Chitedze Biotech Lab	Working order
Nissan Patrol vehicle (BS 4159)	Chassis No.: JN1TCSY61Z058515; Engine: TD42225522	2013	Chitedze	Working order

SECTION THREE: Impacts, success stories and learning outcomes

Impact of CDP in Malawi

Impact area	Impact
How many students were trained by this project directly and indirectly	A total of five students were trained: • Three MSc • Two PhD.
No. of projects using the CDP facilities	Three projects: • IITA-GIZ • ASWAP • AGRA.
No. of students and/or staff using facilities and reagents of CDP	Used by 176 students and staff to carry out their experiments.
No. of people that have been inspired by the project	• Five scientists submitted their research proposals following success stories learned from CDP • Several hundred farmers realized the benefits of obtaining clean seed.
Institutional visibility – recognition of the institute's capacities	• Before the CDP intervention, the molecular biotechnology laboratory did not exist. Since laboratory establishment, LUANAR has been sending students to it • DARS was present at the national agricultural show where it showcased the work done in the laboratory and the new varieties of cassava.
Infrastructural capacity – helping student execute their project	• Well-equipped molecular biology genotyping equipment • Well-functioning tissue culture laboratory and growth room • Three well-equipped Biosafety Level 2 laboratories • Well-equipped molecular diagnostic laboratory • Two screenhouses for insect-controlled experiments • Fast internet facilities • Accessibility to peer-reviewed journals through AGORA, Plant Disease, Phytopathology, Molecular Plant-Microbe Interactions journal subscriptions in 2014–2015 • Bioinformatics facility for sequencing analysis.
New stakeholders interacting with the project	• A number of stakeholders have interacted with the project, including LUANAR.

Service the lab has provided	• A total of 4258 cassava samples were cleaned and indexed from other projects • The laboratory provided research services to MSc and PhD students (for the number of students trained see Table 9).
How many farmers have benefited either directly or indirectly	Training and materials benefited 255 farmers: • CDP trained farmers in good cassava agronomic practices, pest and disease identification and management practices.
New collaborations/collaborative projects	Two new collaborations: • IITA-GIZ on cassava commercialization • ASWAP on the characterization on viral diseases in banana, sweet potato and cassava.
People using information generated by this project	• Extension circulars were provided to farmers and are being used on disease diagnosis and field management • Other projects (Table 7) are using clean seed materials; awareness of these was promoted by CDP.
Benefits to the government – extension training, inspectors and regulators	• Government extension workers benefited from a number of trainings and seminars on field CBSD and CMD diagnostics • Seed inspectors were also involved in seed systems and field identification of pests and diseases.
Advocacy – impacts on policy, etc.	• CDP contributed knowledge that was used in incorporating cassava into Malawi's new National Seed Policy. Previously the seed policy neglected vegetatively propagated crops.
Publications and other communications including other communication materials	• Three scientific papers in peer-reviewed journals • Four conference papers • Three newspaper articles • Two online news stories (see References).
Increase in crop yield and incomes (especially farmers who used clean materials)	• Farmers attributed high yields due to project intervention. The use of clean planting materials, proper phytosanitary measures etc. contributed to the high yields • Some farmers have started cutting cassava as seed. This is a booming business among smallholder farmers where those with large pieces of land and irrigation facilities can make more than MK10 million (US$13,333.00) per season.
Meetings and conferences attended – for the whole team	• Rutgers University Microbiology Symposium, 6–9 February 2017, New Brunswick, New Jersey, USA

	• American Society of Microbiology Microbe Symposium, 1–5 June 2017, New Orleans, Louisiana, USA • International Plant and Animal Genome Conference (PAG XXV), 14–18 January 2017, San Diego, California, USA • 9th Virus Evolution Workshop, 9–11 March 2017, Pennsylvania State University, PA, USA • World Congress on Root and Tuber Crops. January 2016. Nanning, China.
New businesses initiated as a result of this project	• A number of businesses were motivated by the CDP project. The most notable include the seed system business (detailed above), where farmers can make more than US$13,333.00 per season, and the baking industry. Due to high yields, availability of cassava is enabling women to make cassava flour and to produce baked items such as cakes.
Involvement of vulnerable groups	• The project has involved women from the onset. They are among the most vulnerable groups in Malawi. The project has empowered them through a number of businesses as discussed above.
Change of farmers perceptions	A number of perceptions have changed due to CDP intervention. Notable ones include: • Some farmers thinking that CMD is not a disease but rather a nutritional deficiency • The use of clean planting materials as a tool for high yield. Farmers thought that it did not matter what planting material was used, since cassava can withstand 'harsh' environments • Farmers never imagined that cassava seed production could be a viable business. People are used to going to shops to buy well-packed maize seed but never thought cassava planting materials could also be a marketable business.
Other universities requesting to use the facilities/equipment	• LUANAR.
Minimizing/arresting brain drain	• Two postgraduate students and four project staff were employed by the project to work and be associated with project activities in Malawi. This contributed to minimize brain drain in the country.
Building a network of scientists	• The project has collaborated with scientists from other CDP partners in Africa (TARI, JKUAT, RAB, NaCRRI, ZARI and IIAM). Additionally, Rutgers

	University (USA), DSMZ Plant Virus Department (Germany), Makerere University (Uganda), North Carolina State University (NCSU, USA), Department of Agriculture and Food Western Australia and the University of Western Australia (UWA) and Virology Laboratory, CIAT (Colombia).

Success stories

CDP's work in Malawi

The CDP has been operating in Malawi since 2009 based at Chitedze Agricultural Research Station which is part of the DARS in Lilongwe. The project comprised core CDP team members, postgraduate students and government employees who provided laboratory and field support.

Figure 17 Farmers at one of their demonstration plots

Until recently, the only means of deciding if planting material was disease-free was through visual assessment – the lack of visible symptoms signified a disease-free plant. Through this type of assessment, plants have been distributed from one area to another in good faith, only to develop infection at a later stage of growth. This has contributed to the spread of CMD and CBSD throughout the country. The work carried out by the CDP team has contributed to the knowledge and yield of cassava in a number of ways, with the aim of combating the spread of CMD and CBSD.

Education of local farming communities

Early surveys of farmer knowledge about cassava diseases indicated a need for an education program to help farmers recognize the symptoms of viral diseases affecting their crops, and to provide them with the means to access disease-free material. The CDP project organized field days with an emphasis on disease symptom recognition and the use of clean planting material. During one of these events, some 180 visitors (male, female and children) were recorded, reflecting the interest of the farming community in improving their crops. The participation of the project team in agricultural fairs has also been an effective educational vehicle. Posters and plant samples

underlined the benefits of clean planting material. The presence of eminent visitors, such as the President of Malawi and the Chief Justice, helped to disseminate the message at the highest levels.

Detection of cassava viruses and production of clean planting materials

Through the CDP team's research, diagnostics tools have been used to detect the presence of viral diseases at molecular level. This work has enabled researchers to link disease symptoms to specific viruses. This important research step has provided the ability to identify those cassava varieties that are not affected by the known cassava viruses.

These disease-free varieties have been tested at the research facility and proven in the field through the demonstration-plot experiments carried out with the participation of farmers across Malawi. This has been a direct involvement with farmers who have been able to compare the yield of the tested varieties with their own preferred plants. The harvested cassava crop from these demonstration plots was shared among farmers, giving the latter a stock of clean planting material for their next planting phase.

Figure 18 Farmers harvesting cassava at demonstration plot in Karonga 2016 and showing the crop obtained

High yield from improved agronomic practices, pest and disease identification and management

Karonga is a traditional cassava growing area and cassava is one of their main staple foods. Mr Kanyimbo who lives in Vithukutu is one of the lead farmers who hosted the CDP cassava demonstration plot in 2015/16 growing season.

Figure 19 Vithukutu farmer, Mr Kanyimbo, displaying a good harvest of cassava from improved variety disseminated through demonstration fields

Mr Kanyimbo stated that he had been growing local varieties (Matakolembwende and Maso azungu), which were low yielding and prone to diseases. Additionally, they had been using ridges – which were very large and widely spaced – and this contributed to low yields.

Through the CDP, Mr Kanyimbo was trained in good cassava agronomic practices, pest and diseases identification and management practices and he also hosted a demonstration plot. Through that, he was introduced to new varieties of Sagonja, Kalawe and Mpale. The new varieties were tolerant to diseases and high yielding and the kondowole (fermented flour) processed from these varieties is acceptable (nsima is very white and cooked leaves are tasty) which has made his household more food secure.

After training, he adjusted ridge size and spacing, resulting in higher yields. He gave an example that he harvested 3.5 bags of 50 kg of Sagonja compared to 1.5 bags of Matakolembwende from the same area (one ridge of 10 m long). Through the training, he learned pest and disease identification which led him to select planting materials from the nursery farm. Previously, he was just getting the planting materials without any selection. In 2016/17, he further multiplied the materials he obtained from the demonstration plot and managed to sell 450 bundles at MK800 (US$1.10) per bundle – something that had never happened in the past. Being a lead farmer, this knowledge is being shared with other farmers.

Networking

Through the project, DARS has strengthened institutional networks with other project implementing partners within Africa as well as well-known institutions abroad. Among the latter are Rutgers University (USA), DSMZ Plant Virus Department (Germany), Makerere University (Uganda), NCSU, Department of Agriculture and Food Western Australia and UWA and Virology Laboratory, CIAT (Colombia). Such collaborations have raised the profile of the work carried out in Malawi and has

been a source of encouragement for young scientists to work in their own country and know that they can access up-to-date knowledge in their field.

Learning outcomes

Cassava production techniques

The CDP has strengthened farmers' knowledge on cassava production techniques especially on CMD and CBSD identification, management and control. This has significantly increased farmers' cassava yields, sustained their livelihoods and improved their economic strength. This also has promoted several businesses and opportunities for smallholder famers (details under Impacts above).

Diagnostics and control of viral pathogens

The characterization of viruses and disease vectors has improved researchers' knowledge on biology of viruses associated with cassava in the country and beyond. This is ideal for designing detection and diagnostics methods for control of viral pathogens.

Surveillance and monitoring of disease spread

Through the project, it has been established that cassava viruses continue to migrate from one place to another in East and Central Africa. This knowledge has helped scientists and policy makers to strengthen surveillance and monitoring of disease spread. It has also helped in advancing phytosanitary measures when distributing planting materials from one region to another. Furthermore, disease and virus distribution maps, developed during the project, have assisted scientists to make decisions on where to multiply clean seed and where to plant demonstration plots.

Laboratory documentation

The CDP improved our skill and expertise in documenting laboratory experiments. The laboratory technicians now have a well-organized way of documenting their work, unlike previously. This has also been applied to other non-CDP projects/activities currently being implemented in the laboratory.

Conclusion

The project has added to the country's knowledge about cassava viral diseases. We have provided guidelines for breeders, plant pathologists and other stakeholders in the cassava value chain and seed systems with guidelines on selection and deployment of clean cassava planting materials for food security and income generation among smallholder farmers in Malawi. With respect to CBSVs, our studies have contributed to the molecular and epidemiological knowledge of the viruses that cause the disease by establishing their rate of evolution and understanding their migration and phylogeography in East and Central Africa.

Our molecular findings have also highlighted the existence of a distinct subspecies/strain of CBSV, which was tentatively called CBSV-Tanzania. By depositing our sequences in the public domain (GenBank), we have contributed to further studies on CBSV biology and genetics. The findings of our

project have resulted in an increased body of knowledge that can provide an important input into disease control and monitoring programs.

A valuable extension to the work carried out in this project could be the use of deep sequencing techniques to unravel the novel viruses that might exist in cultivated as well as non-cultivated plants and establish evolutionary relationships between those viruses and cassava viruses identified to date.

Publication of research findings

Manuscripts submitted for publication 2018

Mbewe, W., Hanley-Bowdoin, L., Ndunguru, J. and Duffy, S. (2018) Cassava viruses – host jumps, virus recombination, spread in plant material. *Emerging Plant Diseases and Global Food Security* (in press).

Theses submitted 2018

Mtonga, A. MSc Thesis submitted to Makerere University. *Molecular characterization of Geminiviruses infecting cassava in Malawi*.

Manuscripts under internal review 2018

Mbewe, W., Mukasa, S.B., Tairo, F., Sseruwagi, P., Ndunguru, J. and Duffy, S. Evolutionary history of cassava brown streak virus in East Africa.

Mbewe, W., Pankomera, P., Mhone, A., Mwase, R., Benjala, M. Mtonga, A. Chilungo, S. Benesi, I. Sseruwagi, P., Tairo, F. and Ndunguru, J. Occurrence and spread of cassava brown streak and associated viruses in Malawi 2013 to 2015

Mtonga, A.P., Mbewe, W.K., Mukasa, S.B., Benesi, I.R.M., Mhone, A., Sseruwagi, P., Ndunguru, J. and Tairo, F. A review of cassava mosaic disease status and its associated virus species in Malawi.

Mtonga, A.P., Mbewe W.K., Mukasa, S.B., Benesi, I.R.M., Mhone, A., Sseruwagi, P., Ndunguru, J. and Tairo, F. Small RNA deep sequencing-based detection of cassava viruses: First report of Cassava Virus C in Malawi.

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Mbewe, W., Winter, S., Mukasa, S.B., Tairo, F., Sseruwagi, P., Ndunguru, J. and Duffy, S. (2017b). Deep sequencing reveals a divergent strain of Ugandan cassava brown streak virus isolated from Malawi. *Genome Announcements*, 5(33):e00818-17.

Conference presentations

Mbewe, W., Mukasa, S.B., Tairo, F., Sseruwagi, P., Ndunguru, J., Duffy, S. *High rates of evolution and pervasive purifying selection in coat protein gene of cassava brown streak virus.* Rutgers University Microbiology Symposium, 6 – 9th February 2017, New Brunswick, New Jersey, USA.

Mbewe, W., Duffy, S. *Comparative evolvability of plant viruses*. American Society of Microbiology Microbe Symposium, 1 – 5 June 2017. New Orleans, Louisiana, USA.

Mbewe, W., Mukasa, S.B., Tairo, F., Sseruwagi, P., Ndunguru, J., Duffy, S. *Rates of evolution and pervasive purifying selection among Cassava brown streak viruses*. International Plant and Animal genome Conference (PAG XXV), 14 – 18th January 2017, San Diego, California, USA.

Mbewe, W., Mukasa, S.B., Tairo, F., Sseruwagi, P., Ndunguru, J., Duffy, S. *Temporal structure and rates of evolution in the coat protein gene of cassava brown streak virus*. 9th Virus Evolution Workshop, 9 – 11th March 2017, Pennsylvania State University, PA, USA.

Mbewe, W., Tairo, F., Sseruwagi, P., Ndunguru, J., Duffy, S., Mukasa, S.B., Benesi, I., Sheat, S., Koebler, M., Winter, S. *Variability in P1 gene redefines phylogenetic relationship among Cassava brown streak viruses*. World congress on Root and Tuber Crops. January 2016. Nanning, China.

Acknowledgements

This work was supported by the Tanzania Agricultural Research Institute (TARI)–Mikocheni with joint funding from the Bill & Melinda Gates Foundation (BMGF) and UK Department for International Development (DFID) through the 'Cassava disease diagnostics for sustainable productivity in Africa' project (Grant no. 51466). We are grateful to Ms Leonia Mlaki and Mr Deogratius Mark for technical assistance in laboratory molecular sample analysis.

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ZAMBIA

Patrick Chiza Chikoti[1], Mathias Tembo[1], Miriam Chisola[1], Hilda Mwaba[1], Rabson Mulenga[1], Suwilanji Sichilima[1], Mathews Matimelo[1], Joseph Ndunguru[2], Fred Tairo[2] and Peter Sseruwagi[2]

[1] Zambia Agriculture Research Institute, Mt. Makulu Central Research Station, Private Bag 7, Chilanga, Zambia
[2] Tanzania Agricultural Research Institute (TARI)–Mikocheni, P.O. Box 6226, Dar es Salaam, Tanzania

Abstract

The Cassava Diagnostics Project (CDP) project (2008–2018) was implemented in Zambia through the Zambia Agricultural Research Institute (ZARI). In the 10 years of its implementation, several objectives set in three main aims were successful implemented and their milestones accomplished. Four countrywide surveys were conducted in seven provinces. During the surveys, the prevalence of cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) and whiteflies were assessed using a standardized disease surveillance protocol and representative samples collected for laboratory analysis and identification.

Results of field data showed moderated CMD incidence (47%, 51% and 49%) with no significant differences among 2009, 2013 and 2015 surveys, respectively. The severity of CMD symptoms was also moderate with means on the 1–5 severity scale of 3.25, 2.65 and 3.35, respectively. For the first time in Zambia, CBSD was observed in the 2017 survey with incidence of 32% in North-Western Province. Molecular detection and characterization of the representative samples showed that *African cassava mosaic virus* was predominant (65.4%) followed by *East African cassava mosaic virus* (25%) and *Uganda cassava brown streak virus* was the only species causing CBSD, which was closely related to Ugandan isolates by 94% nucleotide sequence similarity. Whitefly biotyping using mitochondrial *COI* gene sequencing showed the predominant species was sub-Saharan Africa 1 sub-groups 1 and 2.

The seed system was supported by enhancing the capacity of the Seed Control and Certification Institute and a private seed multiplication company to certify cassava planting materials. Monitoring of breeders' crossing and yield performance trials enabled screening of 30 parent materials for viruses and their response to CMD.

Outreach activities built the capacity of 430 cassava farmers through training on cassava agronomic and integrated disease management practices. Over 200,000 cassava farmers across the country were reached directly and indirectly by received information on CMD and CBSD through two television programs produced and aired in 2015 and 2016.

The country's capacity to manage virus diseases was strengthened through human resource and infrastructure capacity enhancement. In the 10 years of CDP implementation, a total of six ZARI staff members were upgraded to MSc level and three research assistants retooled on advanced molecular

diagnostic techniques and bioinformatics in short-term tailor-made training. Infrastructure was enhanced through refurbishment of one screenhouse, acquisition of a project vehicle and several items of laboratory equipment and consumables. ZARI's visibility was enhanced through collaboration with project partners in the country and within the region through participation in exchange visits, training visits and in 12 scientific meetings.

Acronyms and abbreviations

ACMV	*African cassava mosaic virus*
CBSD	Cassava brown streak disease
CBSV	*Cassava brown streak virus*
CDP	Cassava Diagnostics Project
CMD	Cassava mosaic disease
CTL	Country Team Leader
EACMV	*East African cassava mosaic virus*
FAO	Food and Agriculture Organization of the United Nations
IITA	International Institute of Tropical Agriculture
JKUAT	Jomo Kenyatta University for Agriculture and Technology
MAP	Months after planting
mtCOI	Mitochondrial cytochrome oxidase gene
NAIS	National Agriculture Information Service
SCCI	Seed Control and Certification Institute
TARI	Tanzania Agricultural Research Institute
UCBSV	*Uganda cassava brown streak virus*
UNZA	University of Zambia
ZARI	Zambia Agricultural Research Institute

Results summary: Zambia

Aim I: Understand the threat from evolving viruses and vectors	
Objective 1: Disease epidemiology	
Disease and whitefly prevalence surveys conducted	• Four countrywide surveys were conducted in 2009, 2013, 2015 and 2017, respectively. • Mean cassava mosaic disease (CMD) incidence (47%) and severity of 3.2 in 2009, 51% and 2.6 in 2013 and 49% with severity of 3.55 in 2015, respectively. • Mean whitefly populations were 3.6, 1.8 and 11.3 in 2009, 2013 and 2015, respectively. • In 2017, cassava brown streak disease (CBSD) was surveyed in 29 fields, with incidence 32.2% and symptom severity 2.3.
Objective 2: Characterization of emerging viruses	
Cassava virus isolates in the project countries sequenced and analyzed	• Cassava brown streak virus (CBSV) isolates collected in 2017 were sequenced. • Sixty samples were partially sequenced for cassava mosaic begomoviruses from 2013 and 2015 surveys.
Cassava virus distribution maps for partner countries, generated (incidence, severity, whitefly, viruses, sat)	• Maps were produced from the surveys of 2009, 2013, 2015 and 2017. These included 100 CMD as well as CBSD incidence and severity maps.
Objective 3: Characterization of disease vectors	
Whiteflies characterized	• Forty samples were *mtCOI*-PCR amplified and sequences.
Potential insect vectors of CBSVs identified (if any)	• No alternative host was found with CBSVs.
Aim II: Support clean seed systems for farmers	
Objective 6: Conventional breeding support	
Breeders' material monitored for disease and indexed for virus (CMBs and CBSVs) load at 3, 6, 9 and 12 MAP	• Thirty parents were screened for CMD and CBSD.
Farmers trained on CMD and CBSD disease symptom recognition and management strategies	• There were 430 farmers trained in the demonstration sites, agricultural shows and disease surveillance surveys and/or workshops or field days during 2013–2017.
Demonstration plots for benefits of using virus-indexed planting materials established on-farm	• Two demonstrations and 45 farmers, extension workers and NGOs trained.

Information materials developed and disseminated	• Three-hundred flyers for CMD and CBSD copies of dissemination materials printed in English and local languages. • Three-hundred brochures disseminated to cassava farmers. • Three radio programs. • Three TV programs. • One documentary. • Eight papers published in peer-reviewed journals.
Aim III: Build sustainable regional capacity	
Objective 10: Strengthening stakeholder linkages	
Awareness on availability of diagnostic capacities created through training and different media	• Three radio programs produced and aired. • Two TV programs. • Two demonstration plots initiated.
Objective 11: Strengthening human capacity and infrastructure	
Human capacity	
Project staff recruited	• Two research assistants. • One Assistant Country Team Leader (CTL). • One vehicle driver. • One MSc student.
PhD and MSc trained on different aspects of cassava virus diseases	• The CTL, and one PhD and one MSc student.
Advanced specialized training and visits for project scientists (1-2 months) conducted	• Dr Patrick Chikoti worked with the Modelling Group in Rothamsted and Cambridge University, UK, to learn how to model disease spread.
Extension workers, crop inspectors and other stakeholders (1 week) training	• Two workshops were conducted and extension officers trained on cassava viral diseases and their management. • Twelve crop inspectors trained in the same workshop.
Infrastructure strengthening	
Greenhouses constructed/renovated	• One screenhouse completed.

Background

Cassava is one of the most important root crops in Zambia. It is the second most important food crop after maize with an annual production of 1,010,298 t/year (FAOSTAT, 2016). An estimated 30% of the 16 million Zambians consume cassava as part of their daily carbohydrate diet. Cassava is mostly grown by small-scale farmers on fields of less than 1 ha and mainly in the cassava belt, which includes Luapula, Northern, North-Western and Western Provinces (Figure 1). The crop is also increasingly important in Central and Lusaka Provinces. Cassava is used in various ways including as raw material for livestock feed and the starch for paper making and brewing. However, its cultivation is hampered by several abiotic and biotic constraints. Abiotic stresses include low soil fertility, acidic and alkaline soils, drought and low temperatures, especially during winter (May–August). Biotic stresses include cassava green mites (*Mononychellus tanajoa* Bondar), cassava mealy bugs (*Phenacoccus manihoti* Matile-Ferrero), whiteflies (*Bemisia tabaci* Gennadius) and termites (*Cubitermes tenuiceps*). Cassava is also affected by diseases, such as cassava bacterial blight (CBB), cassava mosaic disease (CMD) and cassava brown streak disease (CBSD).

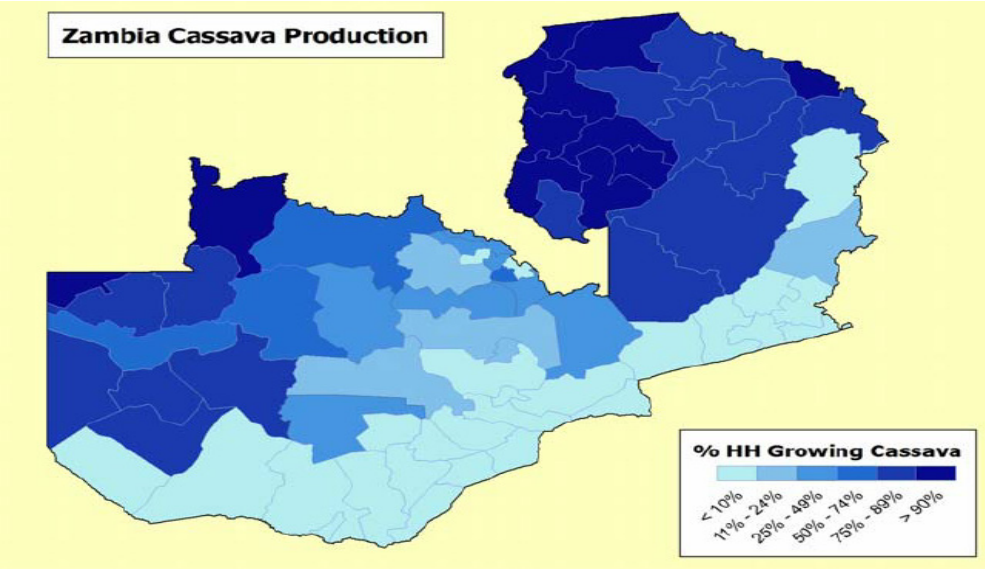

Figure 1 Major cassava-growing zones in Zambia (source Haggblade and Nielsen, 2007)

Whitefly, *B. tabaci*, is the vector of CMD and CBSD. These two diseases are of great economic importance for cassava and cause significant yield losses of over US$1 billion annually worldwide. *Bemisia tabaci* has been associated with CMD in the virus pandemic-affected parts of East and Central Africa (Legg et al., 2014). The CMD is widespread in all key cassava-growing areas of the country (Chikoti et al., 2013) and its incidence was 40.8% in 1996 (Muimba-Kangolongo et al., 1997) and 46% in 2006 (Legg and Chikoti, unpublished). It is currently the most devastating disease of cassava in Zambia (Chikoti et al., 2013) and accounts for 50–70% of cassava yield losses countrywide (Muimba-Kankolongo et al., 1997), with recent estimated annual losses of US$52 million (Tembo et al., 2017). Strategies to control CMD were initiated in the early 1990s and included the release and distribution of CMD-resistant cassava varieties to smallholder farmers. However, efforts to control the disease are limited by lack of sufficient quantities of planting materials of resistant varieties. To mitigate the negative effects of CMD, the Zambia Agriculture Research Institute (ZARI) implemented a ten-year regional project 'Disease diagnostics for sustainable cassava productivity in Africa' that was coordinated by Tanzania Agricultural Research Institute (TARI)–Mikocheni in Tanzania and conducted in seven African countries in east and southern Africa. The project had

three aims: (i) to understand the threat from evolving viruses and vectors, (ii) to support clean seed systems for farmers and (iii) to build sustainable regional capacity. This report presents the activities implemented in Zambia and the achievements during 2008–2018.

SECTION ONE: Understanding the threat from evolving viruses and vectors

Disease epidemiology in Zambia

Four comprehensive field disease diagnostic surveys were conducted in 2009, 2013, 2015 and 2017 in the seven provinces of Zambia. The aims of the surveys were to (1) determine and map the incidence and symptom severity of major cassava viral diseases and abundance of associated insect vectors and (2) determine the geographical distribution of the causal viruses and vectors in the survey areas. The surveys were carried out in Lusaka, Northern, Central, Luapula, Eastern, North-Western and Western Provinces. Cassava leaves with viral disease symptoms were sampled from plants of 3–6 months of age following the standardized survey protocol (Sseruwagi et al., 2017 in the CMD/CBSD survey manual available on the CDP intranet on the Agshare.Today platform).

In addition, asymptomatic cassava leaves were collected in each field to confirm absence of viruses. Sampling was done at intervals of 10–50 km in areas where cassava growing was less intense, but in areas with intense cassava cultivation, fields were sampled at intervals of ≤10 km along main motorable roads. Cutting- and whitefly-CMD infections were distinguished as plants expressing symptoms on all the leaves and those expressing disease symptoms on only the uppermost leaves, respectively.

Cassava mosaic disease

CMD incidence

The overall CMD incidence was 47%, 51% and 49% for 2009, 2013 and 2015, respectively (Figure 2). Generally, the trend of CMD incidence showed the disease was moderate with no significant changes between years. Among provinces, Lusaka was the most affected with CMD incidence increasing from 67% in 2009 to 83% in 2015, followed by North-Western Province with similar increasing incidences across years Although the least affected province was Muchinga Province, in the northeast of the country bordering with Tanzania, the incidence still increased from 27% in 2013 to 30% in 2015. The main source of CMD infection across years and provinces showed that 42.6%, 49.6% and 44.5% in 2009, 2013 and 2015 was cutting borne, and whitefly infection was the least.

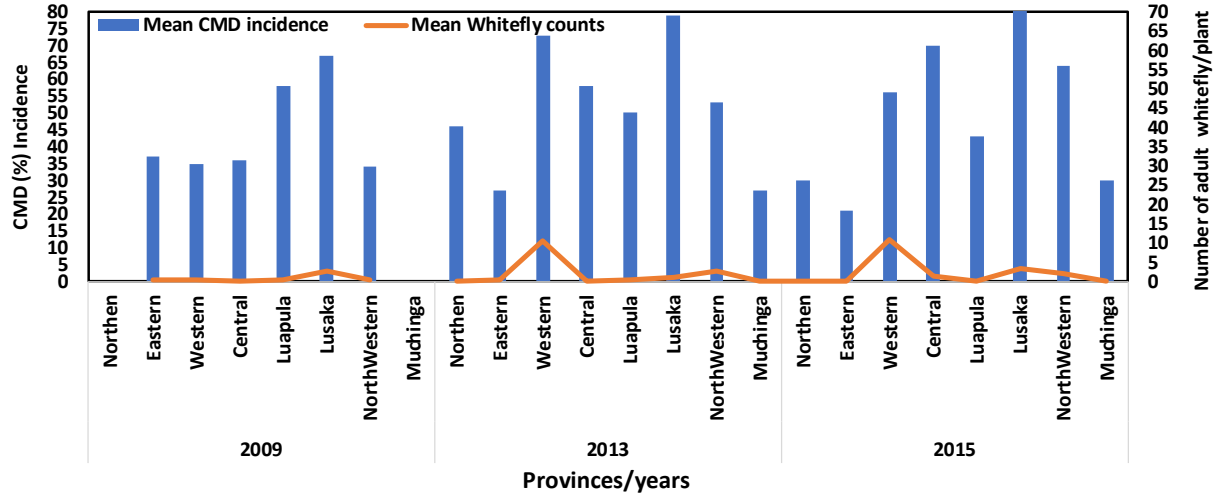

Figure 2 CMD incidence and whitefly abundance trends, 2009–2015

CMD symptom severity

Overall symptom severity across years and provinces was mild to moderate. The CMD symptom severity ranged from 3.2 in 2009 to 3.35 in 2015.

Adult whitefly abundance

Whitefly abundance was low in all provinces. The mean whitefly counts ranged from 0.03 in 2009 to 10.3 in 2015 with slightly higher counts in Western Province both in 2013 (10.5) and 10.8 in 2015 (Figure 2).

During the surveys of 2009–2015, several disease maps were generated and shared with cassava stakeholders in Zambia. The maps were used to enable key stakeholders to make decisions on where to multiply cassava plant materials, and thus govern movement of cassava planting materials within the country. The generated maps include distribution of CMD disease across provinces, the prevalent CMD species and abundance of whitefly (Figure 3 and Figure 4).

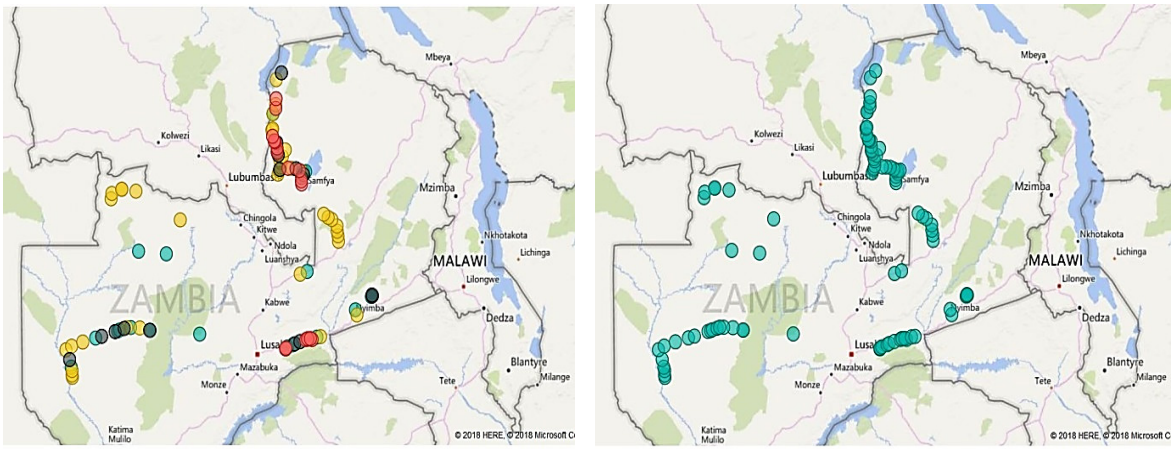

Figure 3 Disease incidence 2009 survey: (left) CMD and (right) CBSD

= 0–25% (Low) >25–50% (Medium) >50–75% (High) >75–100% (Very high)

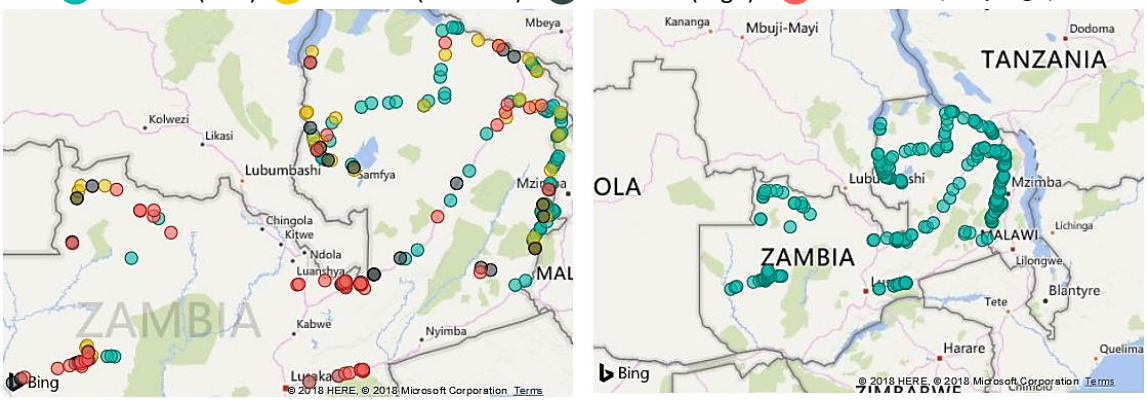

Figure 4 Disease incidence 2015 survey: (left) CMD and (right) CBSD

CBSD incidence

During the surveys conducted in 2009, 2013 and 2015, CBSD was not detected in Zambia. However, in March 2017, CBSD symptoms were observed in North-Western Province. Twenty-nine cassava fields were surveyed and cassava leaf samples collected from 116 plants (92 symptomatic and 24 non-symptomatic). The CBSD prevalence was ~79% (23/29) across fields. Mean CBSD incidence varied across fields but averaged 32.3% and mean disease severity was 2.3 on a 1–5 scale.

Prevalence of improved cassava cultivars

In the surveys conducted, farmers recalled cultivar names, which differed among provinces and years. The most frequently recalled cultivar was Manyokola in all surveys (10 responses) in 2009, 65 in 2013 and 56 in 2015 (Table 1). However, in all provinces and all surveys, the majority of farmers could not recall the name of the cultivar they were growing; therefore, we referred to them as 'unknown'.

Table 1 Predominant cultivars growing in seven provinces in Zambia during 2009, 2013 and 2015 surveys

Cultivar name	2009	2013	2015
Bangwela	6	11	7
Manyopola	10	65	56
Nalumino	14	11	15
Total cultivars recalled	**19**	**39**	**29**

Characterization of emerging viruses

Detection of cassava viruses (CMBs and CBSVs)

CMB analysis

In line with disease surveys, a total of 156 representative cassava leaf samples with CMD-like symptoms were collected for virus detection and genetic characterization to determine genetic diversity of cassava mosaic begomovirus (CMB) species prevalent in Zambia. The PCR results using virus species-specific primers with representative samples from 2009 and 2013 surveys both showed that *African cassava mosaic virus* (ACMV) was the predominant CMB species followed by *East African cassava mosaic virus* (EACMV). Of the 156 tested samples, ACMV was detected in 65.4%, EACMV in 25% and a small proportion of samples (9.6%) were dually infected with ACMV + EACMV (Table 2).

Table 2 Occurrence and geographical distribution of CMBs in seven provinces of Zambia, April–May 2009. Adapted from Chikoti et al. (2013)

Province	CMBs			No. of samples
	ACMV	EACMV	ACMV + EACMV	
Lusaka	13 (52/12.7)	12 (48/30.8)	0 (0/0)	25 (16.0)
Luapula	23 (46/22.5)	17 (34/43.6)	10 (20/66.7)	50 (32.1)
Northern	18 (100/17.6)	0 (0/0)	0 (0/0)	18 (11.5)
North-Western	12 (100/11.8)	0 (0/0)	0 (0/0)	12 (7.7)
Central	8 (80/7.8)	0 (0/0)	2 (20/13.3)	10 (6.4)
Western	16 (84.2/15.7)	0 (0/0)	3 (15.8/20)	19 (12.2)
Eastern	12 (54.5/11.8)	10 (45.5/25.6)	0 (0/0)	22 (14.1)
Totals	**102 (65.4)**	**39 (25.0)**	**15 (9.6)**	**156**

In addition to PCR results, total of 60 PCR products were directly sequenced by Sanger method. Phylogenetic analysis of all the partial sequences with similar sequence identity resulted in four clades that included isolates *ACMV*-UG Mild Uganda (AF126800.1), *ACMV-UG*Svr Uganda (AF126802.1), *ACMV*-[MG:MG310A1] Madagascar and *ACMV*-CM39 Cameroon (AY211462.1)

(Mulenga et al., 2016). The Zambian isolates showed substantial homology with clade members, with sequence identities ranging within 97–98%. Within the *EACMV* species, however, isolates showed greater variability, with a wide sequence divergence (77–99%).

In a separate analysis of 41 core coat-protein nucleotide partial sequences (550 bp), obtained from the 2014 sample isolates (KT869078–KT869118), the Zambian isolates clustered with several global isolates of the seven begomovirus species, albeit several Zambian isolates did not clearly resolve into specific clades (Figure 5A). However, complete DNA-A genome sequences of 19 CMBs (KT869119–KT869131 and KP890349–KP890354) obtained from a selected number of isolates formed elaborate clades with specific groups of virus isolates obtained from GenBank (Figure 5B). Sequence identity among similar species of the Zambian isolates was within 98–100% compared with 95–97% for other members of the phylogroup.

CBSV analysis

Laboratory analysis confirmed the presence of *Cassava brown streak viruses* (CBSVs) in Zambia in a separate field assessment carried out in July 2017 in Luapula (Chienge and Nchelenge districts) and Northern (Kaputa district) Provinces, following observations of plants with CBSD-like symptoms there. Using RT-PCR and sequencing, *Uganda cassava brown streak virus* (UCBSV) was detected in the samples (Mulenga et al., 2018). A comparative analysis of the isolate with the National Center for Biotechnology Information (NCBI) revealed its nature as a sequence variant of UCBSV sharing 94/96% maximum complete polyprotein nt/amino acid identities with isolates from Malawi (MF379362) and Tanzania (FJ039520).

Characterization of disease vectors

Although *B. tabaci* has previously been observed in farmers' cassava fields in Zambia, the population has always been generally low (<1 per plant). However, surveys conducted in Northern, Luapula, Western, Lusaka, North-Western, Eastern and Central Provinces in 2013 and 2015 showed an increase (>1 to 5 per plant) in adult whitefly abundance in some farmers' fields. This was attributed to the presence of the species sub-Saharan Africa 1 sub-group 1 (SSA1-SG1), which is currently associated with high populations in East Africa (Legg et al., 2014). We studied the diversity of *B. tabaci* on cassava in Zambia using specimens collected in the 2015 survey.

A total of 42 samples were used to study the molecular diversity and distribution of *B. tabaci*. The *B. tabaci* sequences grouped within the SSA1 clade when compared with other species in NCBI. Sequence alignment followed by phylogenetic analysis grouped the Zambian sequences in two major clusters, supported by high bootstrap values (>50): SG1 and SG2. The *B. tabaci* samples from Western and North-Western Provinces comprised SG1 and SG2, respectively, whereas most samples from Eastern Province were clustered within SG2.

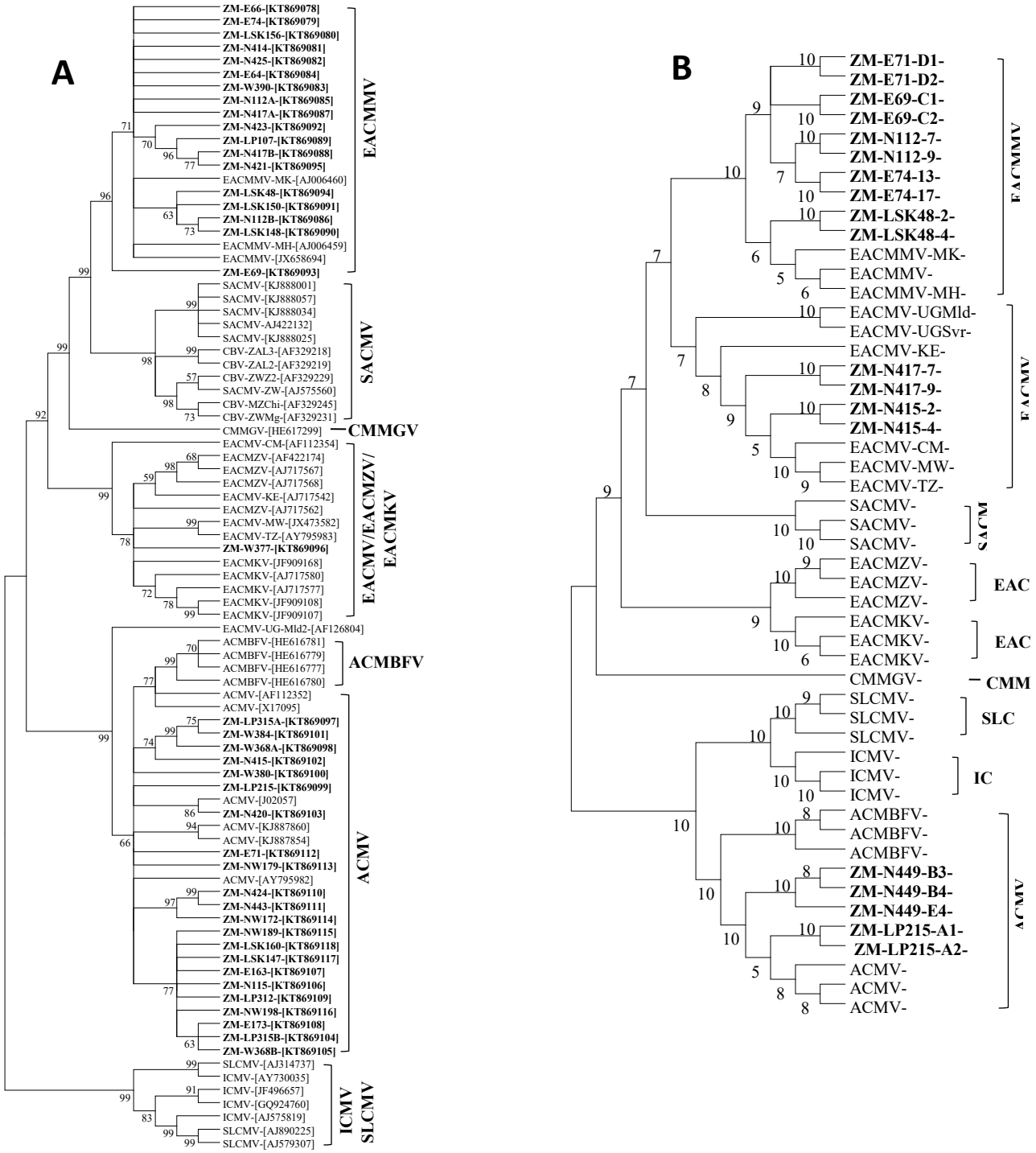

Figure 5 Phylogenetic relationships between cassava mosaic geminiviruses from Zambia (in bold) and global virus isolates based on analysis of aligned core coat protein (A) and complete DNA-A genome nucleotide sequences (B). Adapted from Mulenga et al. (2016)

SECTION TWO: Integrated pest management

Conventional breeding support

Breeders' material monitored for disease and indexed for virus (CMBs and CBSVs)

Among the various strategies for management of CMD, planting virus-free cuttings is the most effective for minimizing disease spread. However, for effective management of CMD, it is impracticable to use a 'one-size-fits-all' strategy. Therefore, the management efforts promoted included supporting clean seed systems for farmers, conventional breeding and reaching farmers directly.

Support clean seed systems for farmers

Supporting certification systems

Figure 6 Seed inspectors at the Seed Inspector Training, Mt. Makulu Research Station, Chilanga (left) and Mansa Research Station (right)

To improve the quality of cassava seed, the Cassava Diagnostics Project (CDP) embarked on training of personnel in charge of regulating the seed industry with advanced skills in recognizing cassava viral disease symptoms. Twelve inspectors, comprising three senior staff and nine technicians from the Seed Control and Certification Institute (SCCI), a government department that regulates the seed industry in Zambia, were trained in 2014 at Mansa Research Station (Figure 6). The training covered the following topics: (i) recognition of CMD and CBSD symptoms, (ii) assessment of disease incidence and symptom severity and (iii) how to collect samples for laboratory testing of virus diseases.

At present there is no formal cassava seed system in Zambia. Although several individual farmers are multiplying and selling cassava planting materials, checking for diseases and the causal viruses is not routine. For this reason, CDP-Zambia monitored the cassava multiplication fields and offered technical backstopping to Arulusa Farm (Figure 7) in Central Zambia, which is approximately 60 km from Lusaka and has 54 ha of cassava grown only for seed.

Following building a successful partnership with Arulusa, CDP-Zambia has provided technical advice on the management of both cassava and sweet potato viruses to the company, which enabled them to maintain a low CMD threshold (4%) in the planting materials they sell.

Figure 7 CDP scientists (Drs Ndunguru, Sseruwagi and Chikoti) visit Arulusa Farm with the General Manager (in checked shirt) to provide technical advice on inspection of plants for cassava viral diseases (left) and the ZARI staff testing of planting materials for CMD status at Mt. Makaula Laboratory in Lusaka (right)

Support to cassava breeders

Breeders' material monitored for disease and indexed for CMBs and CBSVs

Support to breeders was strengthened through increased monitoring of research trials consisting of crossing blocks and preliminary yield trials. These efforts were aimed at creating partnerships and common agreement on CMD evaluation in terms of disease rating. Two cassava breeders within the ZARI, Root and Tuber Improvement Program had their trials evaluated for CMD. The trials included a preliminary yield trial in Mansa (Figure 8), and seedling trials (three sites) in Western, Lusaka and Luapula Provinces.

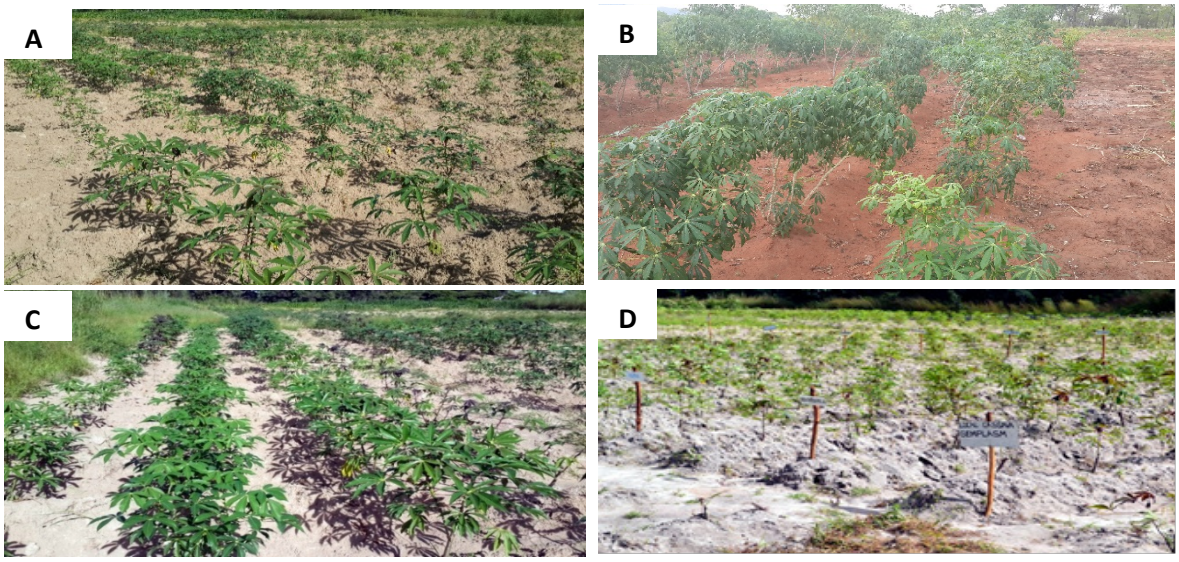

Figure 8 Cassava preliminary yield trials at Manza (A and B) and Rufunsa (C and D) assessed by CDP

Reaching farmers directly and through partners

Interaction with cassava farmers during sample collection in field surveys

Since 2013, ZARI scientists have interacted directly with farmers during biannual surveys in their cassava fields. A total of 430 farmers were directly contacted: 180 in 2013 and 250 in 2015 (Figure 9). Farmers were asked what cultivars they grew and what they knew about cassava pests and

diseases, specifically CMD, CBSD and whiteflies. The interaction lasted 10–20 minutes per farmer. Regardless of whether they knew CMD or CBSD, the farmers were educated on how to recognize the diseases. In addition, management strategies were also highlighted to farmers. The farmers were also given brochures and leaflets as extra information (Figure 9). The brochures were more pictorial for easier understanding.

Figure 9 Researchers interacting with farmers directly during cassava diagnostic surveys in Mansa district, Luapula Province

Farmers trained on CMD and CBSD disease symptom recognition and management strategies

Reaching farmers through agricultural shows

One mode of information dissemination to the cassava community in the project was through participating in district and national agricultural shows. Participation in the shows aimed to (1) inform the general public about the CDP, (2) educate farmers on how to recognize and manage cassava diseases, particularly CMD and CBSD, and (3) showcase different technologies that ZARI is developing. More than 6500 farmers passed through the agricultural show stands at district, provincial and national shows during 2013–2017 (Figure 10). Among the high-ranking show participants who visited the stand in 2016 at the National Agricultural and Commercial Show included Mr Roland Msiska (Secretary to the Treasury), Ms Dora Siliya (then Minister of Agriculture), Mr Shawa (Permanent Secretary for Agriculture) and The Honourable Davis Mwila (Minister of Defense). The exhibits included healthy and CMD-infected plants (Figure 10), cassava roots and information products on cassava disease management (leaflets and brochures). More than 1000 information products were given out to farmers and those with an interest in growing cassava.

Figure 10 Visitors to the ZARI stand in 2017: (A) CDP Assistant Country Team Leader (Assistant CTL, Mathias Tembo, in light blue suit) showing the Secretary to the Cabinet, Rolland Msiska, (B) CDP Assistant CTL showing the Minister of Agriculture, Dora Siliya (left), Minister of Defense, Davies Chama (second left) and the Deputy Director exhibits (C) of CMD-infected cassava and tissue culture plantlets and (D) harvested stool of cassava from the virus-free improved cassava variety

Training of women farmers

A training workshop on 'Cassava Diseases and Management' was held at Mount Makulu Research Station, Lusaka, in 2016, under the auspices of the Plant Pathology Unit. The workshop was attended by 22 women farmers growing cassava from Chilanga district (Figure 11). The criterion used in the selection of participants was that the women were required to have a field of cassava, so that techniques learned could be immediately put into practice. The participants were trained on cassava diseases and their management. A participatory framework was created, and the participants had the opportunity to present their views and knowledge on cassava management and challenges. The challenges were discussed during the plenary session and advice was offered by experts.

Figure 11 Cassava farmers attending training at Mt. Makulu Research Station on cassava diseases and management in 2016

The workshop was structured around two main processes. The first was an experience sharing process, mostly theory, on the first day – this included group discussions and presentations by the participants. The second day consisted of field visits, in which participants were presented with diseased samples and asked what they thought about the cassava diseases.

The participants were of the view that such training should continue and be given more time, especially with regard to symptom identification and management of cassava diseases. In addition to identification and management of cassava diseases, the participants and facilitators agreed to consolidate the partnership for the farmers to realize their full potential in cassava production.

Information materials developed and disseminated

Information products on CMD and CBSD were produced for various stakeholders. The materials were in the form of brochures and leaflets (Table 3). Over 10,000 of the information products were distributed to farmers during 2013–2017.

Table 3 List of information products developed under the CDP 2013–2017 on cassava diseases and their management

SN.	Material	Topic	Type	Target audience	Quantity issued
1	Brochure	Recognition of CMD and CBSD and management	3	Farmers, extension agents	5000
2	Leaflet	Recognition of CMD and CBSD and management	2	Farmers, extension agents	5000
3	Radio messages	About CDP, importance of cassava diseases and how to manage them	2	Farmers, extension agents	3
4	TV interviews	Importance of CMD and CBSD in Zambia, outbreak of CBSD in Zambia and interventions required	3	General public (farmers, extension agents, policy makers)	3
5	TV documentary	About CDP, importance of cassava diseases and how to manage them	1	General public (farmers, extension agents, policy makers)	1
6	Prevalence maps	Distribution and incidence and severity of CMD in Zambia	2	Seed multipliers, seed regulator, farmers, extension agents	100
7	Research journal articles	Distribution and incidence and severity of CMD in Zambia		General public (farmers, extension agents, policy makers, scientists)	9
8	Advisory note	Spread of CBSD in Zambia	1	Policy makers	1

Cassava documentary

A documentary on cassava was produced in September 2016 in partnership with the National Agriculture Information Service (NAIS). The NAIS is a Department within the Ministry of Agriculture and is responsible for disseminating agriculture information to the farming community. The

documentary covered (i) an overview of the CDP, (ii) how to recognize CMD and CBSD symptoms and (iii) the importance of managing CMD and CBSD.

Figure 12 NAIS personnel with Zambia CTL Dr Patrick Chikoti (A and B) during the production of CDP documentary at Three Sisters Farm, Rufunsa, in 2016

During production, one of the demonstration sites (Three Sisters Farm, Rufunsa) was used to highlight the CDP activities (Figure 12). The documentary was produced by a public broadcaster, the Zambia National Broadcasting Corporation, and was broadcasted in all 10 provinces of Zambia. The same documentary was also aired by the two privately owned Muvi and Prime TV stations which target mainly major towns. Each channel made more than one broadcast and over 200,000 members of the public viewed the documentary. The estimated viewership was based on NAIS projections.

Demonstration plots for benefits of using virus-indexed planting materials established on-farm

On-farm demonstration plots were established to show the benefits of growing virus-indexed cassava planting material compared with unindexed material. The demonstrations were established at three sites: Rufunsa on the Three Sisters Farm (Figure 13), Kaoma on Shandy's Farm, and Mansa on the Mansa Research Station. Rufunsa and Kaoma are located in region II and experience annual rainfall of 800–1000 mm. Mansa is in agro-ecological region III, and has annual rainfall of >1000 mm. The farmers were also trained in good agricultural practices, including the following:

i) benefits of using clean indexed planting material
ii) planting density
iii) weeding
iv) recognition and management of CMD and CBSD
v) scouting for CMD and CBSD.

In all three sites, planting to harvesting was done with the farmers. More than 60 farmers benefited directly by learning how to plant, recognize and manage CMD, CBSD and CBB. These demonstration plots provided farmers with skills that will enable them to increase productivity in their cassava fields.

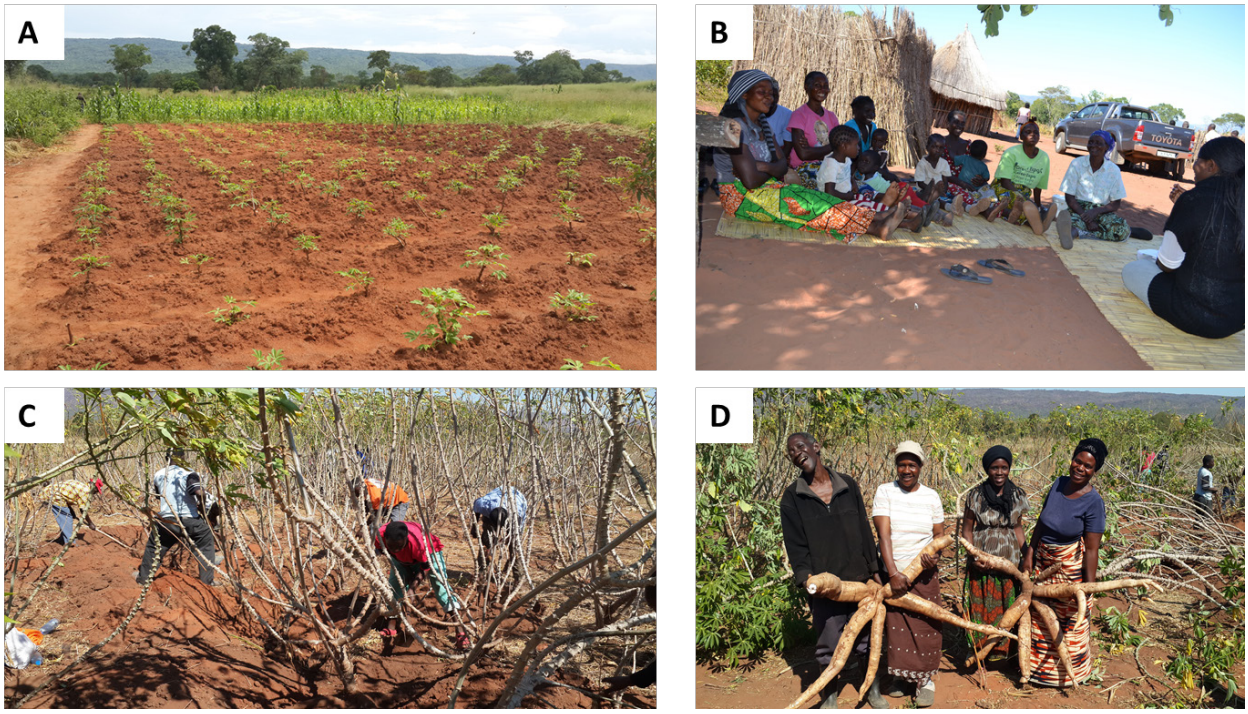

Figure 13 CDP activities in Rufunsa at Three Sisters Farm: (A) a cassava field planted with disease-free cassava stems, (B) CDP Research Assistant gathering farming practices from the farmers, (C) farmers harvesting cassava from the demonstration plot and (D) farmers showcasing their harvested cassava from the demonstration plot planted using virus-free improved cassava

Strengthening stakeholder linkages

Project inception and consultative meeting with stakeholders

During the first phase, one awareness meeting was held at Annias Lodge in Chilanga. Among the organizations represented were ZARI scientists, senior extension officers, seed regulators and a Food and Agriculture Organization of the United Nations (FAO) representative (Table 4 and Figure 14). The objective of the meeting was to introduce the CDP to the partners that were working on cassava-related activities.

Table 4 List of participants to the inspection workshop

SN	Name of Participant	Organization	Position	Gender
1	Dr Kamona	ZARI	Director	Male
2	Dr Joseph Ndunguru	TARI-Mikocheni	Director	Male
3	Mrs Monde Zulu	ZARI	Chief Agriculture Research Officer	Female
4	Mr Mulonga	FAO		Male
5	Mr Isaac Sichilima	SCCI	Seed Inspector	Male
6		NAIS		
7	Mr Arundile Sakala	ZARI – Plant Quarantine and Phytosanitary Service	Principal Agriculture Research Officer	Male
8	Mr Kenneth Msiska	ZARI – Plant Quarantine and Phytosanitary Service	Principal Agriculture Research Officer	Male

SN	Name of Participant	Organization	Position	Gender
9	Dr Chiona	Ministry of Agriculture – Extension Service	Principal Agriculture Research Officer	Male

Before the start of the second phase, further awareness about the project was made at the University of Zambia (UNZA) and ZARI – Mt. Makulu Research Station. At UNZA the awareness meeting was attended by lecturers from the School of Natural Sciences, including Professors Kapooria, Nkunika and Mbata, and Drs Kaimoyo and Katongo. At Mt. Makulu, awareness was increased of researchers from the Plant Protection and Quarantine Division.

Figure 14 Participants at the first phase project awareness meeting, Chilanga, Zambia in 2010

Stakeholders engagement

Since the CDP inception, the Zambian team has developed local partnerships in Zambia. To consolidate the partnerships, the project helped to build capacities through various training sessions. The institutions whose capacities were increased included UNZA, Mulungushi University, SCCI, Arulusa Farm and the Extension Service (Ministry of Agriculture). The skills enhanced included identification of CMD symptoms and laboratory molecular techniques (Table 5 and Figure 15).

Table 5 Local partners and the capacities increased by CDP in Zambia 2013–2016

SN	Institution	Skill	Number of personnel
1	UNZA	Molecular techniques	6
2	Mulungushi University	Molecular techniques	4
3	SCCI	Molecular techniques, CMD identification, management strategies	12

SN	Institution	Skill	Number of personnel
4	Extension Service (Ministry of Agriculture)	CMD identification, management strategies	20
5	NAIS	Production of TV and radio documentaries on project activities	2
6	Arulusa Farm	CMD identification, management strategies	1

Figure 15 Partnering with stakeholders: (A) Mr Mathews Matimelo in Katete district, Eastern Province training farmers on management of plant diseases, (B) extension officers having hands-on in a farmer's cassava field in Kaoma district, Western Province, (C) extension officers in a training workshop in Kaoma and (D) CDP scientists examining cassava planting material at Arulusa Farm

Table 6 List of partners and stakeholders

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
ZARI	Chilanga	NARS (Project partners)	Dr Patrick Chikoti (CTL), Mr Mathews Matimelo (Assistant CTL)	Hosting of the project and CMD diagnostics	Dr Patrick Chikoti, Mr Mathews Matimelo
ZARI	Chilanga	NARS	Dr Mweshi Mukanga (Chief Agric Research Officer), Ms Mulonda Sakajila (Technical Research Assistant), Mr Mathias Tembo (Senior Agric Research Officer), Ms Judith Malumo (Agric Research Officer)	Research and improvement of cassava	Dr Mweshi Mukanga
ZARI	Chilanga and Misamfu	NARS	Ms Maimuna Abass Luangala (Agric Research Officer), Dr Martin Chiona (Cassava Breeder)	Plant health, and breeding for CMD resistance and general improvement	Ms Maimuna Luangala, Dr Martin Chiona
Zambia Agricultural Research Institute	Chilanga	Collaborator	Mr Ivor Mukuka (National Coordinator) Cassava Mechanization and Agro-Processing Project (CAMAP)	Processing and value addition	Mr Ivor Mukuka
International Institute of Tropical Agriculture (IITA), Zambia	Lusaka	Collaborator	Dr Pheneas Ntawuruhunga (Project Coordinator), Feed the Future Project	Breeding and promotion of cassava to improve food security	Dr Pheneas Ntawuruhunga
SCCI	Chilanga	Seed Certifier	Mr Richard Chanda (Principal Seed Officer and Biotechnology Department Head)	Seed certification and control	Mr Richard Chanda
UNZA	Lusaka	University	Dr Langa Tembo	Training and supervision of students	Dr Langa Tembo
Arulusa Farm	Chisamba	Private seed producer	Mr John Kimani (Farm Manager)	Production of cassava seed materials	Mr John Kimani
Farmers	Kalundu, Rufunsa District	Farmer	Ms Margaret Kamusasa, Ms Monica Chitabala, Ms Agness Mukoma and Mr Damus Lungu	Cassava production	Ms Margaret Kamusasa

Exchange visits between scientists in the project countries

One scientific exchange visit between project countries was held in Zambia during 16–20 May 2016. The aim was to share and exchange views and ideas on project operations. Among the participants were the CTLs and the project management teams from Tanzania, Malawi, Kenya, Rwanda, Uganda and Mozambique (Figure 16). During the scientific exchange visit, a field visit was made to farmers' fields in Rufunsa, 160 km from Lusaka (Figure 16). In addition, a visit was made to the UNZA, a key local partner of the CDP ZARI team. Dr Joseph Ndunguru made a key note address on the project overview and achievements. From the exchange visit the scientists learned:

i) how Zambia was closely working and sharing information with cassava farmers in Rufunsa
ii) how the project was utilizing infrastructure such as screenhouses and laboratory facilities in understanding the viruses affecting cassava in Zambia. Within the first year of the project Zambia had built the screenhouses and procured the laboratory equipment.

Figure 16 (A) CDP scientists with farmers in Rufunsa, (B) scientists looking at a cassava plant infected with CMD at Three Sisters Farm in Rufunsa, Lusaka Province

Hosting of cassava diagnostic annual meeting

Zambia hosted the 7th Annual Meeting of the Disease Diagnostics for Sustainable Cassava Productivity in Africa Project at Radisson Blu Hotel, Lusaka, during 10–12 October 2016. The meeting was attended by 58 participants from the UK, Australia, USA and Africa.

The objectives of the meeting were to:

i) review research activities for the past year and plan future activities
ii) share scientific knowledge generated from project activities
iii) strengthen existing collaboration with scientists from different projects working on cassava.

The meeting was officially opened by Mr Moses Mwale, Director of ZARI. In his address, he thanked the Project Managers for convening the meeting in Zambia. He also applauded the Zambia CDP team for their many successes.

In his introductory remarks, the Project Coordinator, Dr Joseph Ndunguru, reminded the participants of the significance of the project. He stated that availability of reliable and affordable capacity to diagnose CMBs and CBSVs accurately and effectively in the major cassava-growing countries is a key to effective management of CMD and CBSD. He further mentioned that through the collaboration, robust standardized molecular diagnostic tools for sensitive and rapid detection of viruses infecting cassava had been developed and shared among scientists in the network countries. Virus characterization and disease surveillance were conducted in all project countries, generating both

disease prevalence and virus distribution maps that are being used for informed decision making especially by extension agents.

Cassava virus disease epidemiological modelling: a visit to Rothamsted and University of Cambridge (UK)

Dr Patrick Chikoti and Professor Elijah Ateka [Jomo Kenyatta University for Agriculture and Technology (JKUAT)] visited Rothamsted Research and Cambridge University to learn how to model disease spread. The key objectives of the study visit were to (i) develop and advise on sampling strategies for CBSD in Zambia and (ii) develop predictive models for CBSD spread to new areas. Thus, while in the UK, the disease surveillance and whitefly abundance data collected in 2009 and 2013 were utilized to model disease spread in unsampled areas and predict the future spread of disease and identify where, when and how disease monitoring programs should target areas where cassava viruses have been detected or areas likely to be affected.

A sampling strategy was developed with Rothamsted Research and used in the 2015 countrywide survey (Anna et al., unpublished). Using the designed strategy, CBSD was detected in the 2017 survey in Luapula (Chienge and Nchelenge districts) and Northern Provinces (Kaputa district) (Mulenga et al., 2018). The other significant outputs of the four-week study visit that contributed to the CDP success were (i) sampling protocols and methods strengthened, (ii) intervention strategies for monitoring and mitigation and (iii) knowledge sharing and transfer between collaborating institutions which resulted in the contribution to publication of a paper 'Spatial Dynamics and Control of a Crop Pathogen with Mixed-Mode Transmission' and 'Advice for cassava brown streak disease (CBSD) sampling strategy in Zambia'.

Technical backstopping

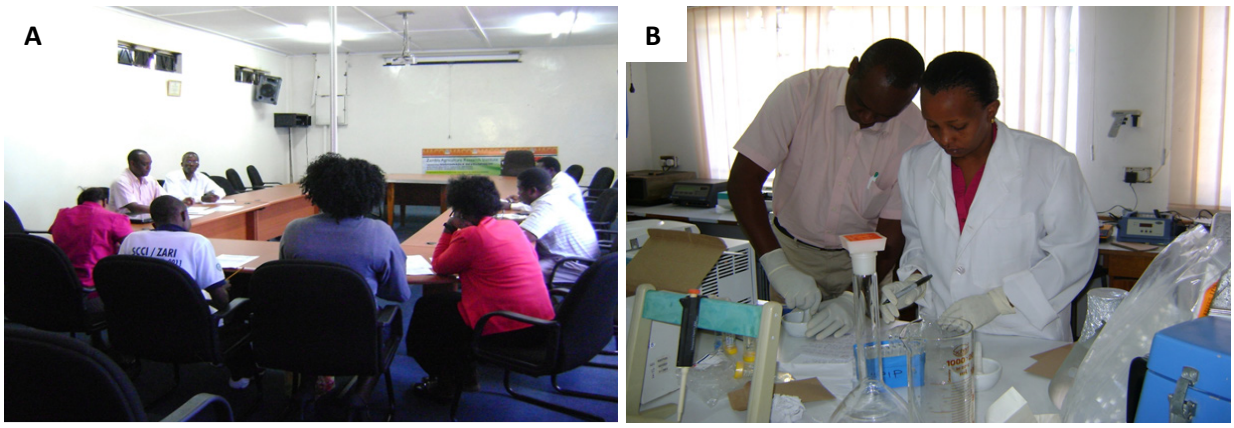

Figure 17 Technical back stopping: (A) members of CDP-Zambia conferring with Dr Fred Tairo at Mt. Makulu and (B) Dr Fred Tairo and Ms Catherine Gwandu training Zambian CDP research assistants on molecular diagnostic method for cassava viruses at Mt. Makulu Laboratory in 2014

In 2014, scientists from TARI-Mikocheni visited ZARI for technical backstopping in deep sequencing methods for virus detection and characterization. The objective of the visit was twofold: (i) familiarization with CDP-Zambia operations and (ii) imparting knowledge on deep sequencing to Zambian scientists. The visit involved collection of diseased cassava samples from the field and laboratory extraction of nucleic acids and analysis using a deep sequencing technique (Figure 17).

Strengthening human capacity and infrastructure

Prior to commencement of project activities, a team of scientists was constituted from among the members of ZARI with the exception of research assistants (Table 7).

Table 7 List of project staff recruited

No of staff recruited	Position	Name
1	CTL	Dr Patrick Chiza Chikoti
1	Assistant CTL	Mr Mathias Tembo
1	Research Assistant	Mrs Miriam Chisola Goma
1	Laboratory Technician	Ms Sichilima Suwilanji
1	Driver	Mr Vincent Mwanza

PhD and MSc trained on different aspects of cassava virus diseases

Six students (five MSc and one PhD) received support either directly or indirectly through use of CDP project equipment and reagents in their research studies (Table 8). To date, four students have graduated.

Table 8 List of students trained under CDP-Zambia

S/No.	Name	Research topic	Degree*	Status
1	Rabson Mulenga	Multiplex polymerase chain reaction for the simultaneous detection of CMBs in Zambia	MSc – full support by CDP	Completed
2	Mathias Tembo	Molecular epidemiology of CMD in selected cassava-growing provinces of Zambia	MSc – partial support by CDP	Completed
3	Tiuzeyani Zulu	Characterization of cassava (*Manihot esculenta* Crantz) varieties resistant to Cassava mosaic virus in Zambia	MSc	Completing in 2018
4	Batisba Tembo	Development of wheat (*Triticum aestivum*) germplasm resistant to spot blotch disease for summer rain-fed conditions in Zambia	PhD	Completed
5	Joseph Banda	Response of sweet potato to sweet potato viruses on beta carotene content, yield and yield components	MSc	Completing in 2018
6	Siankuku Munsaka	Molecular characterization of selected rice varieties in Zambia	MSc	Completed

*Degree program and nature of support

Training of technicians from local partner institutions

The training workshop on 'Cassava Viruses: Biology, Diagnostics and Management' was held at Mount Makulu Research Station, Lusaka, during 6–10 February 2012 (Figure 18), under the auspices of ZARI and IITA. The workshop was attended by 14 participants from UNZA, SCCI and ZARI. The topics included:

i) cassava pests
ii) cassava viruses, symptoms and epidemiology
iii) CMD incidence and severity assessments
iv) collection of diseased samples
v) nucleic acid extraction
vi) analysis of PCR products and interpretation of results.

Training outcome

Most of the participants felt that strong collaboration was required, and that ZARI needed to come up with training materials suitable for farmers to understand and recognize CMD symptoms both in the multiplication plots and farmers' fields. The participants suggested that future training should be given more time, especially concerning symptom identification and management of cassava diseases. In addition to identification, testing and management, the participants from SCCI suggested that the certification protocol for cassava multiplication fields required revision.

Figure 18 Participants from local research institutions attending a one-week training course at the virology laboratory at ZARI in collaboration with IITA

Bioinformatics training of CDP staff in Kenya

The CDP staff from Zambia for bioinformatics training included Mr Mathias Tembo, Mr Rabson Mulenga and Ms Miriam C. Goma. The training workshop (Figure 19) 'Bioinformatics Tools for Species Identification' with special focus on whitefly and CBSD genomic datasets was held during 7–10 June 2016 at JKUAT, Kenya. The workshop was hosted by Dr Laura Boykin and Prof. Elijah Ateka. The training focused on how to use programs such as Geneious (alignment and basic analyses of NGS data), JModelTest (determining correct model of evolution of the dataset), PAUP (generating a robust phylogeny) and TextWranger (Mac) or Notepad++ (Windows) to enable analysis of multiple sequence alignments (whiteflies and cassava viruses), interpretation of phylogenetic trees, identifying species of viruses and vectors and designing diagnostic primers based on genetic variability. The training contributed to analyzing the generated virus subsequences and three papers have been published (see 'Publications and conference participation' in Section 3).

Figure 19 (A) Participants at bioinformatics tools for species identification training at JKUAT, Kenya, and (B) Laura Boykin (facilitator) with participants

Data entry and assembly training in Tanzania

The CDP conducted a 'Data Entry and Assembly Workshop' for the surveys conducted in the CDP participating countries since 2009 from 28th November to 2nd December 2016. The workshop involved participants from Kenya, Malawi, Mozambique, Uganda, Zambia and Tanzania (Figure 20). Zambia was represented by Mr Mathias Tembo and Ms Miriam C. Goma. The workshop was hosted by Dr Peter Sseruwagi, Dr Fred Tairo and Dr Joseph Ndunguru of TARI-Mikocheni and facilitated by Dr Anna Szyniszewska from Rothamsted Research and Mr David Godding from the University of Cambridge, UK. The purpose of the workshop was to ensure that all paper forms from the previous CDP surveys of 2009–2015 were scanned and digitized for easier analysis and retrieval. The participants were trained on coordinated entry and general techniques of scientific data entry. Metadata were also collected on the exact protocol used for the different surveys. The objectives of the training were to:

i) maximize the amount of verified correct survey data
ii) establish links between digitized survey forms and original paper survey forms
iii) train data entry clerks to independently enter data to specified standards.

The 2009–2017 survey data are now accessible through Agshare.Today for participating partner countries under the CDP project. Agshare.Today is a platform in which information is shared among project members.


Figure 20 CDP staff having data entry discussions (left), David Godding, facilitator, scanning hard copies in Dar es Salaam, Tanzania in 2016 (right)

Infrastructure strengthening

To support implementation project activities, several pieces of infrastructure were either procured or refurbished.

Motor vehicle

Prior to the second phase (2008–2012), the Plant Pathology Team did not have reliable transport to carry out the project activities including procurement, disease surveillance and collection of samples for laboratory analysis. The vehicles that were then available were constantly breaking down and contributing to delays in implementing project activities. In the second phase of the project, a brand-new Toyota Hilux was procured.

Screenhouse

One screenhouse was constructed at ZARI with the support of the CDP. The screenhouse is used for biological studies to maintain field-collected cassava plants with unique symptoms for further study.

Laboratory equipment

To achieve the set objectives of the project, i.e. surveillance of insect pests and diseases and virus and vector identification, various items of equipment were procured during 2009–2016 (Table 9).

Table 9 CDP equipment procured during 2015–2016

S/N	Structure	Equipment	Serial No.	Number	Status
1	Vehicle	Toyota Hilux 4WD double cabin	ALP 5476	1	Functional
2	Greenhouse equipment	Ventilation system	N/A	1	Functional
3		Watering system	N/A	1	Requires some repairs
4	Diagnostic laboratory	Clifton water bath	85610	1	Requires some repairs
5		Stuart BioCote vortex mixer	R000107349	1	Functional

S/N	Structure	Equipment	Serial No.	Number	Status
6		Hettich refrigerated centrifuge (MIKRO 200)	MIKRO 200R D-78532	1	Requires some repairs
7		Hettich micro-centrifuge	MIKRO 120	1	Functional
8		Ultra-freezer (–20°C)	Bosch	1	Functional
9		Techne PCR Machine	TC-5000	1	Requires some repairs on the display panel
10		Stuart hotplate stirrer	SB 302	1	Functional
11		Stuart stirrer	SB 161	1	Functional
12		Thermal shaker	TS-100	1	Functional
13		Geno grinder	MINI-G 16002; SN: 10091	1	Functional
14		PCR workstation (UVT-8AR)	SN: 0401071606086	1	Functional
15		PIKO 96 thermal cycler	PK961300715	1	Functional
16		VWR electrophoretic tank	SN: 700-0056	1	Functional
17		VWR electrophoretic tank	700-01136/30044283	1	Functional
18		VWR electrophoretic tank	700-0136/30044280	1	Functional
19		Laminar flow		1	Functional

Tissue culture facility

A new facility was established at Mt. Makulu with funds obtained from the Finnish Government. The facility has attracted much interest from various stakeholders. The facility is now being used for micropropagation for the rapid generation of 'clean' plantlets for a number of running and upcoming project activities. The projected increase in cassava demand among small and emerging farmers and the need to produce clean planting material cannot be overemphasized. The demand for clean planting material is projected to increase because of industries being set up to produce beer, starch and ethanol. One such project is the 'Establishment of a decentralized and sustainable pipeline for the production and deployment of disease-free cassava planting materials in Zambia (2016–2020)', being spearheaded by ZARI. The equipment in the virology laboratory procured under the CDP has the capacity to index cassava plantlets and other crops.

SECTION THREE: Impacts, success stories and learning outcomes

Impacts

For the duration of the project, several impacts have been registered, including strengthening human and infrastructure capacity, generating information on disease spread and building new linkages with local and international organizations.

Impact area	**Impact**
No. of students trained by this project directly and indirectly	For long-term training (2–4 years), one MSc student was trained directly and five students (one PhD and four MSc) received indirect training. Twenty-seven students received short-term training (up to 2 weeks).
No. of projects using the CDP facilities	Five projects currently use the greenhouses and laboratory equipment (see section on equipment).
No. of students and/or staff using facilities and reagents of CDP	Five ZARI staff members and three students (UNZA and University of KwaZulu-Natal) are currently using the CDP facilities.
No. of people that have been inspired by the project	More than 100 individuals – from UNZA, the National Institute for Industrial and Scientific Research (NISIR), Mulungushi University, Copperbelt University, the National Biosafety Authority and SCCI – have been inspired through the level of investment and the results generated (see sections for infrastructure and publications).
Institutional visibility	Locally and internationally, ZARI's increased standing in terms of credibility and diagnostic capacity has improved. This can be attested in the joint supervision of students and use of the facilities by individuals from other organizations including University of KwaZulu-Natal, UNZA, IITA and Mulungushi University.
Infrastructural capacity – helping student execute their project	With the available infrastructure, seven students received local long-term training.
New stakeholders interacting with the project	Two: National Biosafety Authority and NISIR.
Service the lab has provided	GMO detection (one), indexing of sweet potato (one) and cassava viruses (one).
People using information generated by this project	Extension agents are using information to advise farmers on disease management, and college and university students are using it for their research purposes.

Benefits to the government - extension training, inspectors and regulators	The knowledge generated has enabled extension agents to utilize information on CMD and CBSD occurrence, and how to manage the two diseases.
Advocacy-impacts on policy	One advisory note on the spread of CBSD in Zambia was drafted and submitted to the Ministry of Agriculture. The government is now putting in place stringent measures to contain the disease.
Publications and other communications including other communication materials	Information products including brochures, leaflets, journal articles and DVDs on awareness were produced and distributed. The information material is being utilized by different stakeholders.
Meetings and conferences attended – for the whole team	Several meetings and conferences were attended, and project activities presented at these fora.
Support to breeders and other projects	Support to breeders was provided in form of joint scoring of breeders' trials: crossing blocks, yield trials and parent trials.
Other universities requesting to use the facilities/equipment	Mulungushi, Copperbelt and Zambia Open Universities and UNZA.
Building a network of scientists	With the setting up of CDP a good network of scientists locally and internationally has been built including Dr Evans Kaimoyo (UNZA), Professors Susan Seal and John Colvin (University of Greenwich), Professor Chris Gallighan (University of Cambridge), Professor Van den Bosch (Rothamsted Research) and Dr Laura Boykin (University of Western Australia).

Success stories

ZARI has research stations in all provinces of Zambia and includes Mount Makulu Central Research Station. This is the hub of crop research innovations in Zambia, covering many aspects of crop husbandry. This research station has specialized laboratories in soil and water management and plant protection. The Plant Protection Unit hosts the Plant Pathology Section from where the CDP was administered in Zambia. The plant pathology laboratory has been the central laboratory providing crop disease diagnostic services to farmers and research units in Crop Improvement and Agronomy as well as Plant Protection and Quarantine.

Prior to CDP's introduction in 2009, and the successor project in 2013, diagnosis of crop diseases of virus etiology was largely based on symptomatic determination and to lesser extent enzyme linked immunosorbent assays (ELISA). This was because (i) the laboratories had few and incomplete pieces of equipment for molecular-based detection and identification of pathogens affecting crops and (ii) human capacity and skills to diagnose the diseases using molecular-based tools were limited. This hampered efforts to generate technologies suited for managing crop diseases. With the advent of the CDP, the situation changed. The laboratory has a complete molecular workbench necessary to conduct advanced molecular studies. Using such molecular techniques, the laboratory has identified

specific species of CMBs and CBSVs affecting cassava in Zambia. Before the CDP, CMD was only detected through foliar symptoms. To augment the equipping of the laboratory, several laboratory techniques and laboratory safety training sessions were conducted involving 22 project and non-project staff at various levels. Training in practical laboratory skills has increased the competence of ZARI staff to identify different pathogens by molecular means and they can now conduct research previously deemed impossible.

With the increased human and infrastructure capacity, the laboratory is now collaborating with UNZA and Copperbelt and Mulungushi Universities, who have sent their students and laboratory technicians for training in molecular-based diagnostics. Using the same facilities and competencies attained during the duration of the CDP, we were able to identify ACMV, EACMV, *East African cassava mosaic Malawi virus*, *East African cassava mosaic Zanzibar virus* and *South African cassava mosaic virus* from samples collected countrywide. Further, we reduced the turnaround time between sample collection and dissemination of results to stakeholders from the previous 10 days to 2 days depending on availability of required laboratory reagents.

A total of 20 extension staff were trained in CMD symptom identification, most of whom could not identify CMD symptoms prior to the CDP project. This has potential to increase awareness of CMD beyond the reach of research staff thereby making farmers aware of the negative effect of the disease on yields. Further, through establishing demonstration plots, targeted farmer training and interactions with farmers during surveys, more than 2000 farmers were reached and information on CMD and its management shared. Additionally, two TV and three radio programs were aired on four channels with national coverage. The additive effects of these efforts are now evident. Farmers who previously could not differentiate a diseased from a healthy plant can now do so, and some are practicing disease management. This resulted in a marginal decline in CMD incidence across Zambia during 2013–2017.

Capacity has also been built in scientific communication through training in scientific writing and communication. The positive impact of the training is evident from the publications produced within the duration of the CDP project. Overall, the CDP project has increased the research capabilities of ZARI researchers, and this is beneficial to the country both in the short and long term. The positive impact of the CDP project will have lasting impact on the Zambian farming community and improve livelihoods of the Zambian populace in more ways than one. For Zambia, CDP was a success and contributed greatly to the economy.

Learning outcomes

During the duration of the project, lessons were learned either through day-to-day operations or through training provided by TARI-Mikocheni.

Procedures

Laboratory procedures for handling of samples, analysis and data storage have been enhanced. The procedures in handling of samples included traceability from field to results. We learned that obtaining good results does not only depend on getting to the laboratory but also on the procedures that all the team players follow.

Sharing knowledge

We learned that sharing information with all key stakeholders including farmers, seed regulators, university students and extension agents is key to changing people's perceptions of cassava

diseases. Prior to the start of phase two, very few farmers knew what CMD was in Luapula Province (Chikoti et al., 2016) and certainly not how to manage the disease. With the generation of information, and sharing it with farmers and extension agents, knowledge of the farmers concerning CMD has greatly improved. However, how packaging of information was critical. For farmers, simple illustrations in the form of leaflets with pictures of diseased plants helped considerably and this may have contributed to reduction of CMD in Zambia from highs of 52.0% in 2009 (Chikoti et al., 2013) to 48.9% in 2015 (unpublished).

Training at all levels

Training at all levels is key to meeting set objectives especially with the CDP. Empowering project implementers with necessary skills is critical in achieving targets. Under the CDP, we learned that if you train people charged with implementing the project it is easier to register successes. Equally if the recipient of the information is adequately trained then there is more chance that the information received will be put to good use. One example was during each round of surveys in Zambia, farmers were trained for 10–15 minutes on symptom recognition of CMD and CBSD and how to manage the disease.

Tasking responsibilities

Previously, the CTL was responsible for most of the activities under the CDP and this contributed to slow completion of targets. After attending the leadership training in Uganda in 2015, the CTL changed the approach to tasking responsibilities equitably among team members. For this to happen, it was ensured that all members were competent in their assigned area through in-house training or training organized by TARI-Mikocheni. This new approach saw the team achieving targets on time and more efficiently. From this, the team learned:

i) efficiency was achieved by sharing responsibilities
ii) a sense of belonging for each team member.

Challenges

Surveys – owing to Zambia's land area of 752,000 km^2, conducting surveys in one month proved difficult. Consequently, the surveys generally took more than three months. In addition, surveys were often conducted during the rainy season as this is the only time when disease symptoms are fully expressed on cassava plants. Zambia experiences one rainy season during November–April and the roads can become impassable especially in the countryside. Depending on weather conditions, on a day when it was raining, not more than three fields could be assessed; however, if it was not raining then 8–10 fields could be assessed.

The other challenge was that if the owner of a field was available then the more time was spent assessing the field because, after collecting data, the farmer was interviewed on what he or she thought about diseases. The scientists could then advise the farmer on how best to manage the crop concerning CMD.

Conclusions

The CDP in Zambia has scored many successes as highlighted in this monograph. The successes include investment in infrastructure both human and physical. The investment in this area has contributed to detection of cassava viruses in more detail than previously possible – the first time for

Zambia. As a country, we now know where different virus species are found. This is important as organizations supporting cassava production are now using this information to distribute clean materials, as opposed to buying infected materials from farmers and distributing the same. The seed regulator (SCCI) has also strengthened its capacity for seed inspection of cassava because of receiving direct training from CDP. Most importantly, CDP enabled close interaction with key stakeholders – mainly farmers and extension agents, local universities and international research institutes (IITA and Rothamsted Research) on joint research. Despite these successes there are still more challenges. The detection of CBSD in Zambia poses a major threat to cassava production for small-scale farmers. More and urgent investment is needed to contain the disease if the farmers are to remain productive. A formal seed system in cassava needs to be developed to ensure that farmers engaged in cassava production use clean seed as planting material. Awareness of CBSD needs to increase to reach all local partners: extension agents, farmers, NGOs and seed multipliers.

Publications and conference participation

In order to share the results generated by CDP-Zambia with the local and international scientific community, several articles have been published. The articles mainly focused on viruses and disease vectors occurring in Zambia. These include:

Chikoti, P.C., Ndunguru, J., Melis, R., Tairo, F., Shanahan, P. and Sseruwagi, P. (2013) Cassava mosaic disease and associated viruses in Zambia: occurrence and distribution. *International Journal of Pest Management*, 59:63–72.

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Acknowledgements

This work was supported by Tanzania Agricultural Research Institute (TARI)–Mikocheni with joint funding from the Bill & Melinda Gates Foundation (BMGF) and UK Department for International Development (DFID) through the 'Cassava disease diagnostics for sustainable productivity in Africa' project (Grant no. 51466).

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Tanzania Agricultural Research Institute (TARI)–Mikocheni
P.O. Box 6226, Dar es Salaam, Tanzania

Phone: +255 222700552
Fax: +255 222775549
E-mail: cmmikocheni@tari.go.tz

ISBN 978-9976-59-336-5