TANZANIA

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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 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.

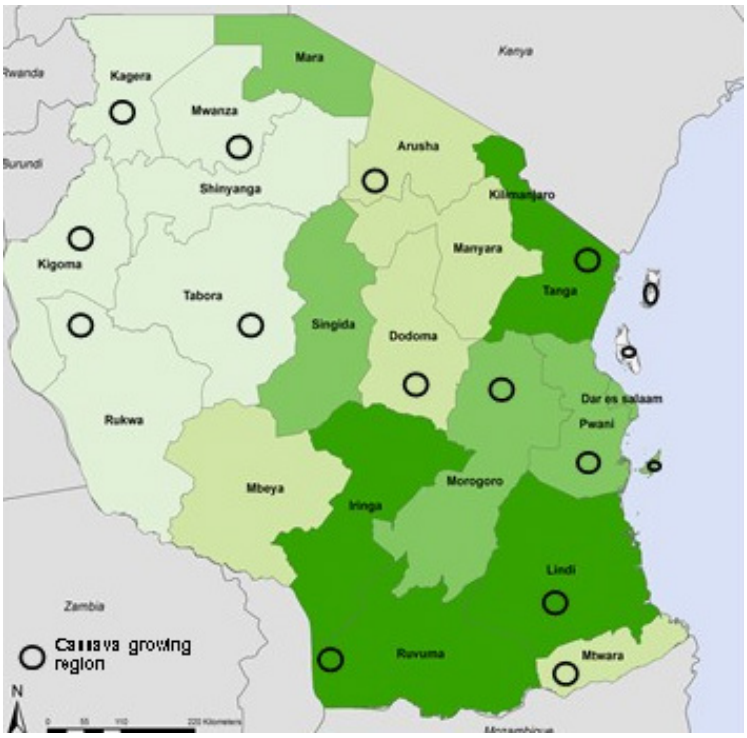

Figure 1 United Republic of Tanzania (Photo source: NBS, 2015), map adapted to illustrate major cassava cultivating regions (circles) within administrative regions (shaded) of Tanzania

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.

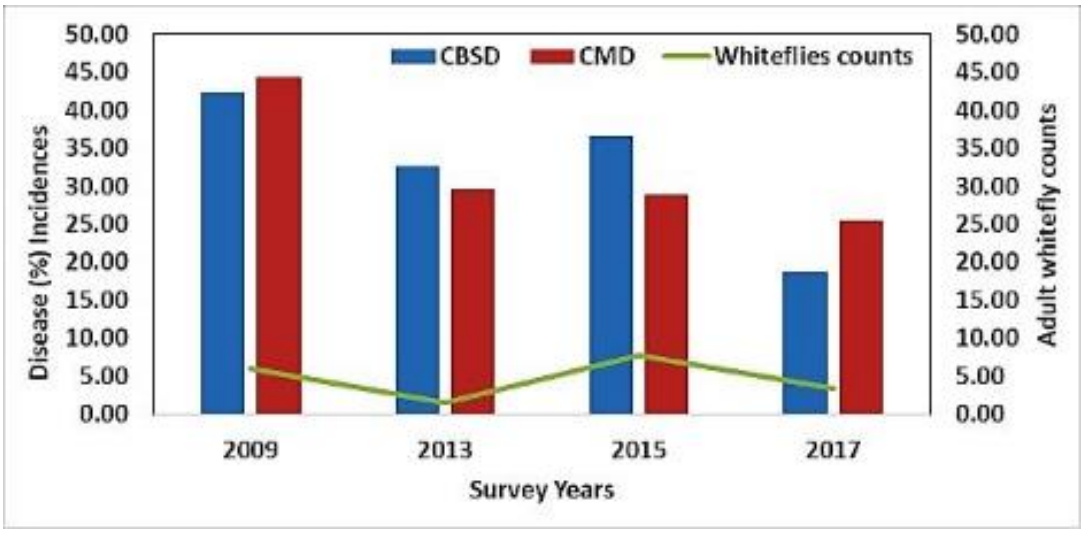

Figure 2 CMD and whitefly abundance trends, 2009–2017 surveys

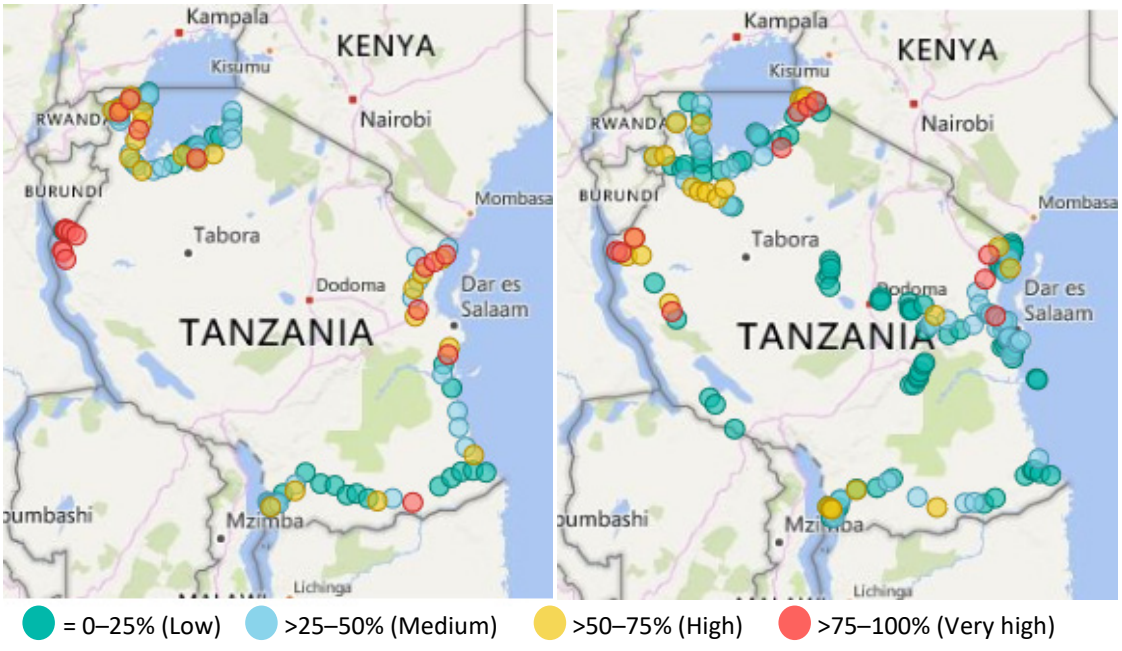

Figure 3 CMD incidence in 2009 (left) and 2015 (right) survey

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).

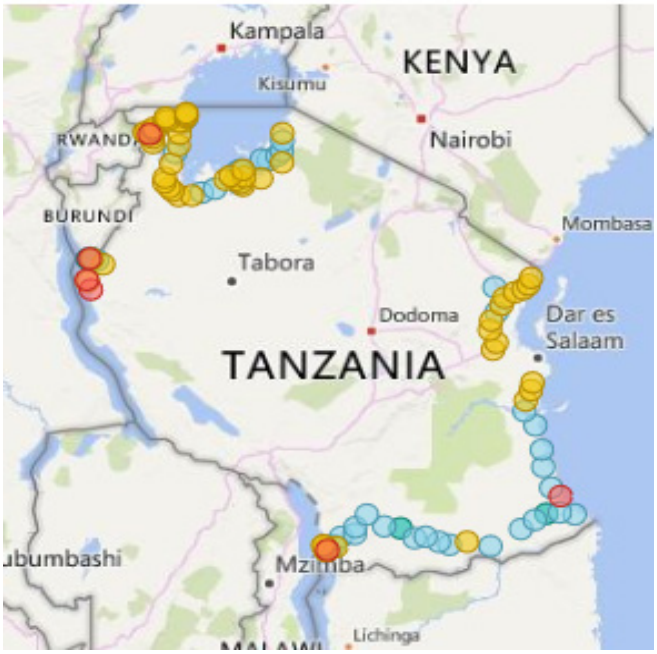

Figure 4 CMD severity map – 2009 survey

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).

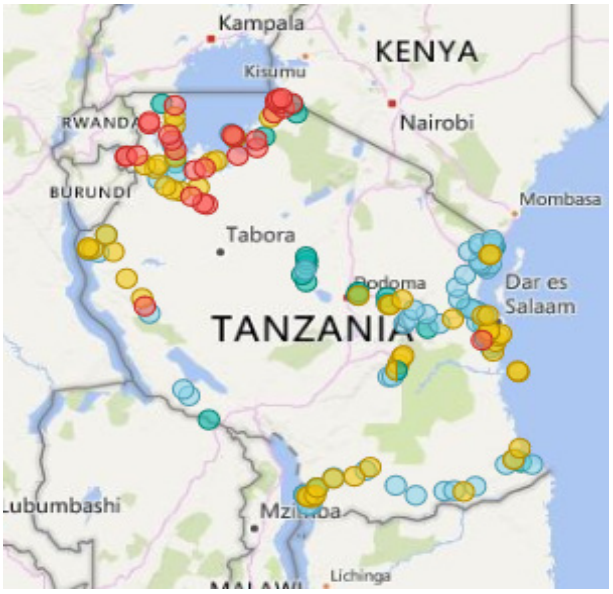

Figure 5 CMD severity – 2015 survey

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).

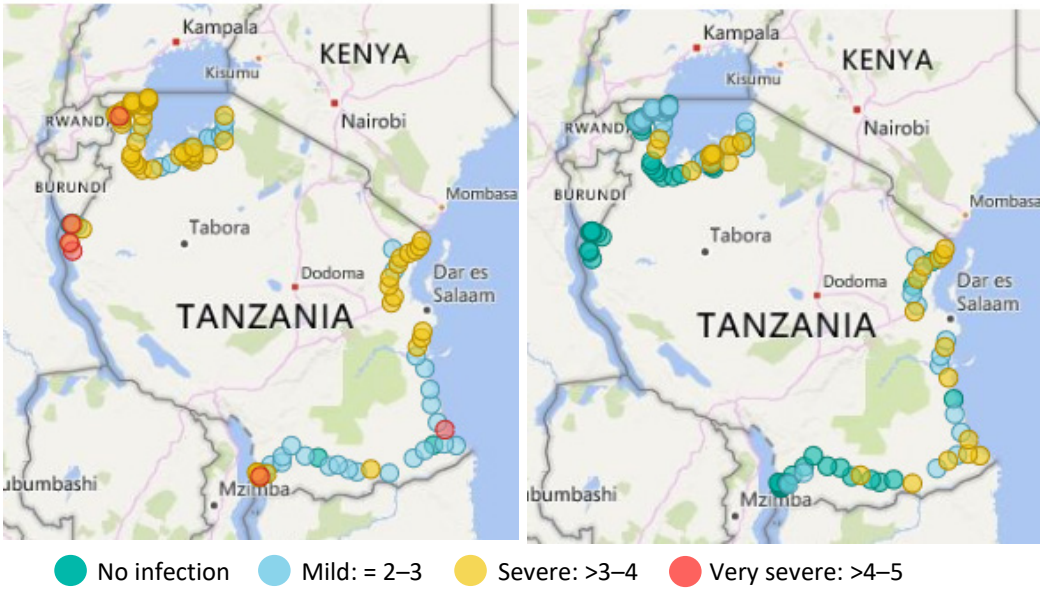

Figure 6 CMD and CBSD severity – 2009 survey

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.

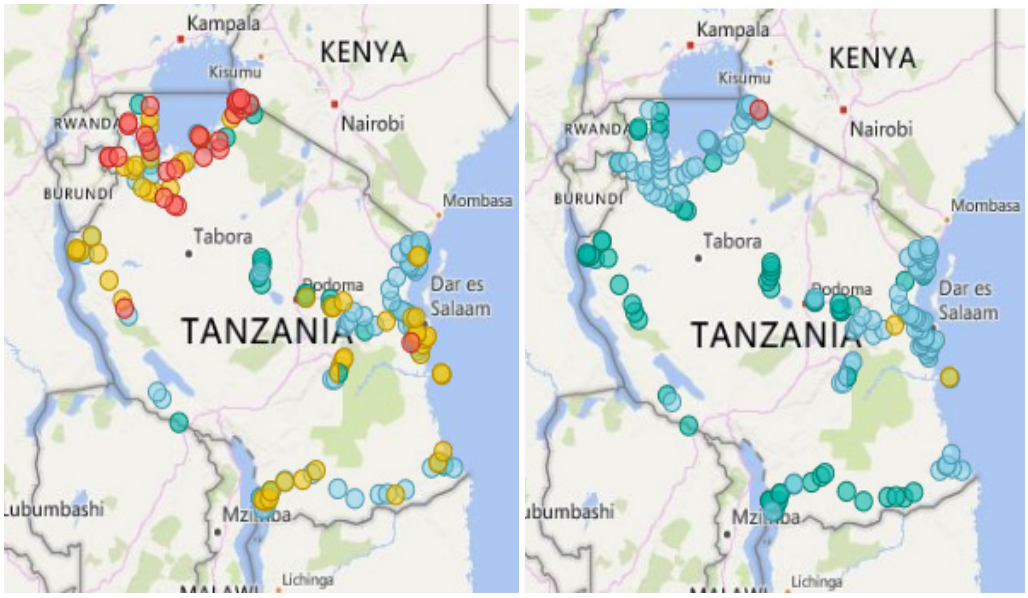

Figure 7 CMD and CBSD severity – 2015 survey

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.

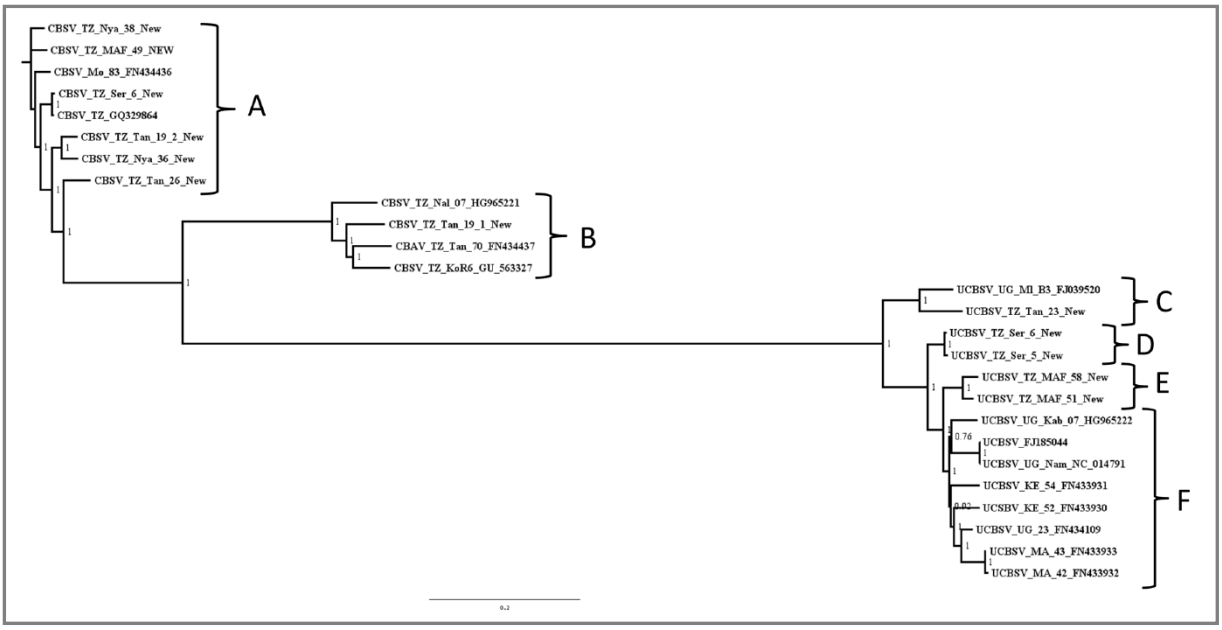

Figure 8 Whole CBSV/UCBSV genomes (nt) analyzed revealed existence of another subclade B with predominantly CBSV isolates from Tanzania (Source Ndunguru et al., 2015)

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.

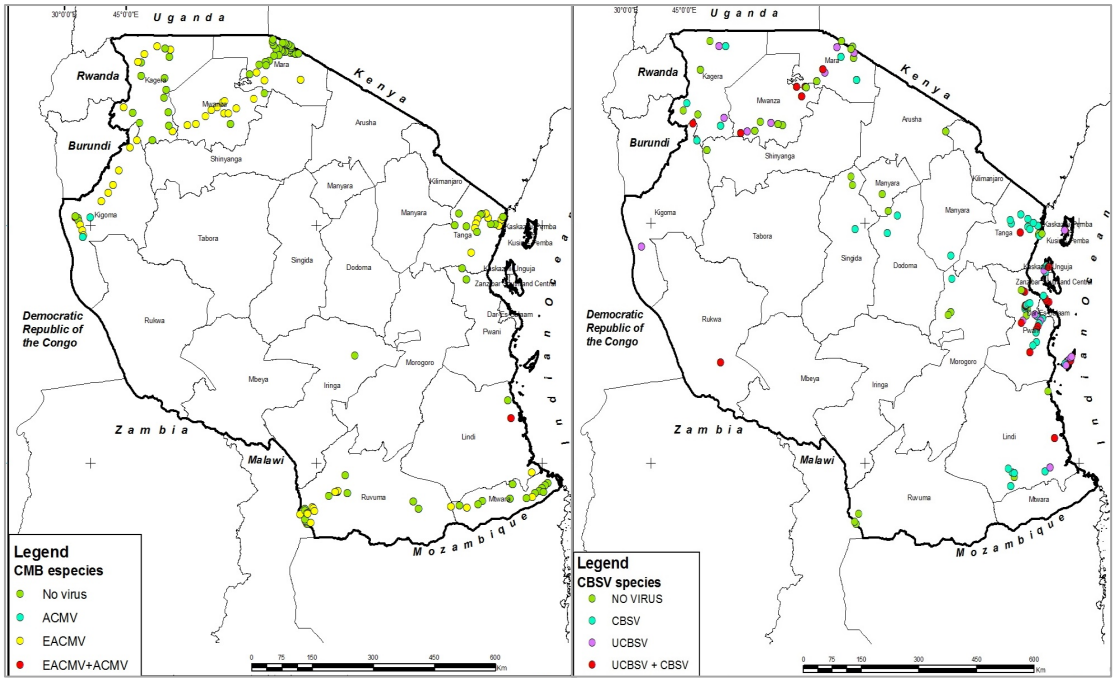

Figure 9 Distribution of viruses affecting cassava in Tanzania: left (CMBs) and right (CBSVs) from the surveys 2013

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.

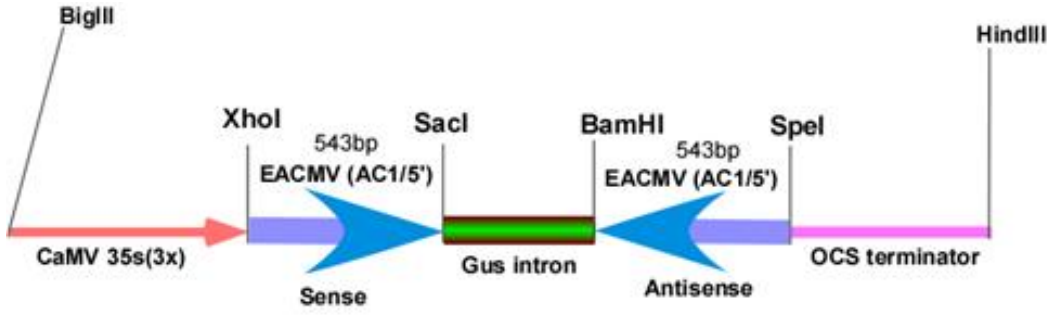

Figure 13 Structure of the molecular construct for EACMV carrying two genes used for cassava transformation to confer resistance against EACMV

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.

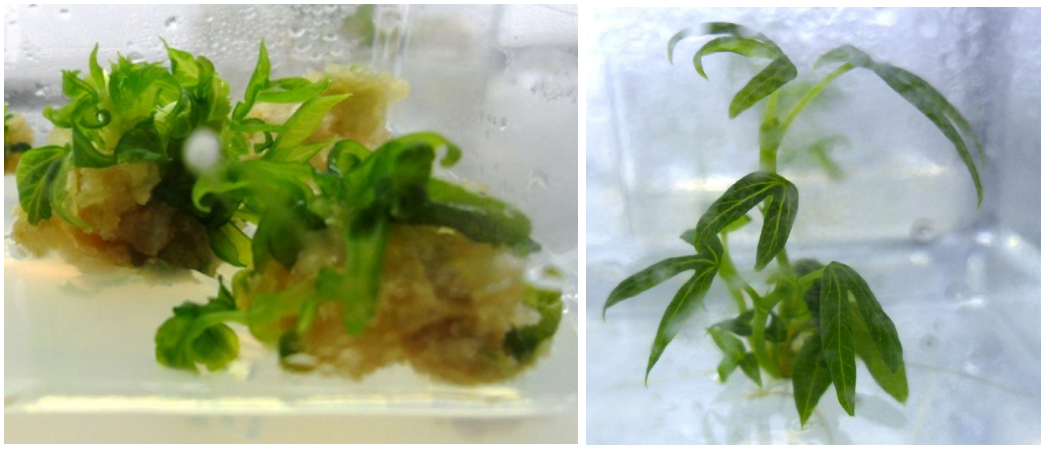

Figure 14 (left) TMS 6044 cassava plants emerging from *GUS*-transformed FEC; (right) TMS 6044 complete cassava plants

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.

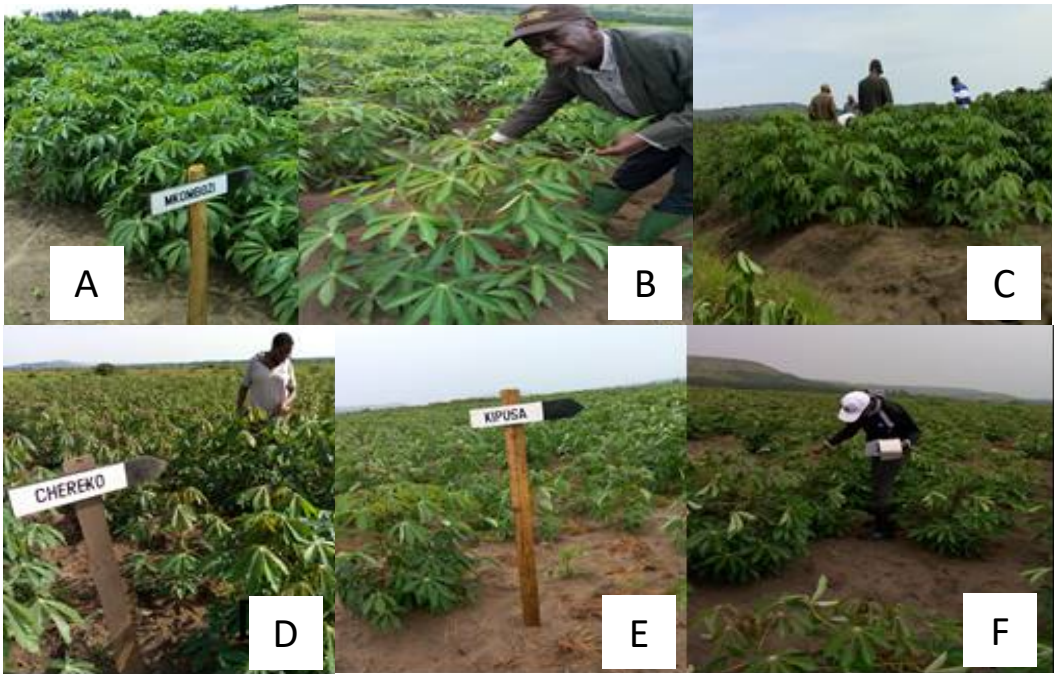

Figure 16 Monitoring and mapping CBSD in multiplication fields in Chato, Muleba, Kisarawe and Mkuranga districts

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 Kebby 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**

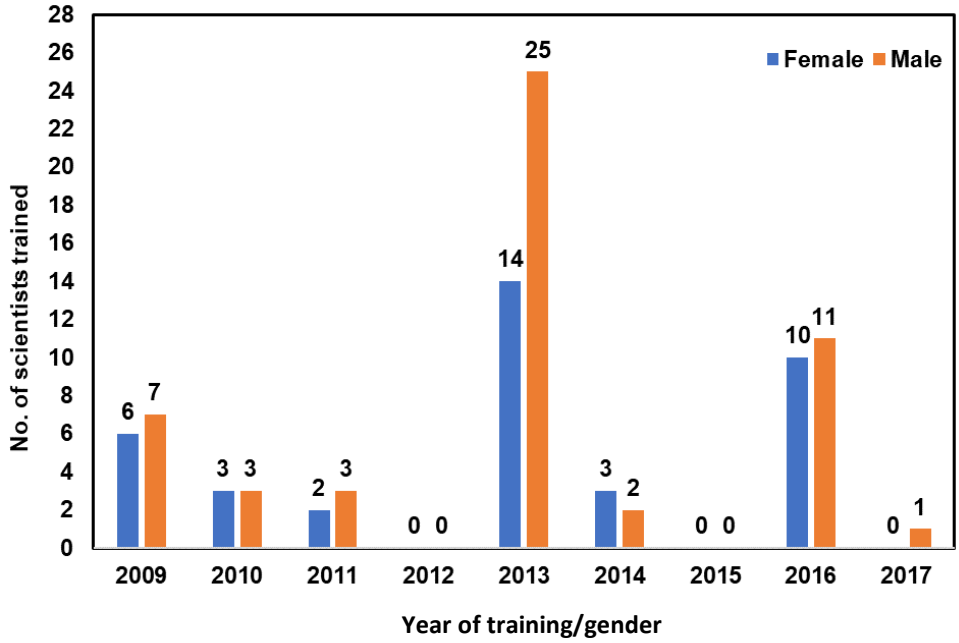

Figure 20 Summary of TARI–Mikocheni scientists (by gender) trained during 2009–2017

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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