MALAWI

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Abstract

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

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

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

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

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

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

Acronyms and abbreviations

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

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

Results summary: Malawi

Aim I: Understand the threat from evolving viruses and vectors

Objective 1: Disease epidemiology

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

Objective 2: Characterization of emerging viruses

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

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

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

Background

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

Figure 1 Map of Malawi showing districts

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

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

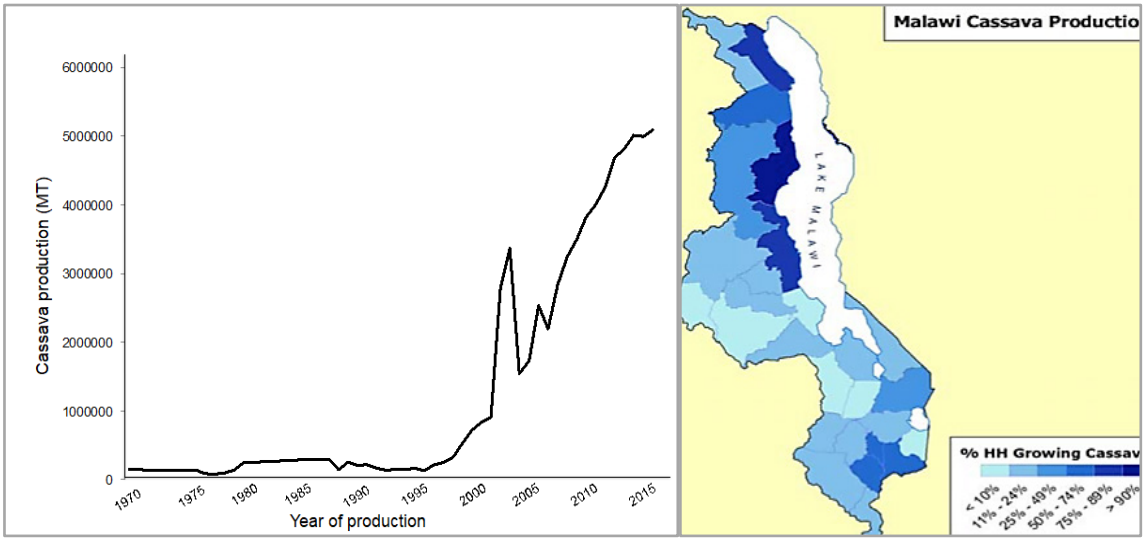

Figure 2 (left) Cassava production trends: 1970–2015. Source: FAOSTAT, 2016 and (right) cassava cultivation areas in Malawi

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

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

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

SECTION ONE: Understanding the threat from evolving viruses and vectors

Disease epidemiology

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

In the 2013 country-wide survey, conducted from 20 July to 6 August 2013, a total of 119 fields were surveyed for CMD and CBSD incidence and severity and adult whitefly populations. A total of 259 leaf samples were collected for analysis in the laboratory: 75 for CMD, 175 for CBSD evaluation and nine for uncultivated plants. Only 94 of the 175 CBSD samples collected were analyzed, 81 samples having deteriorated.

In the 2015 survey, carried out from 25 June to 10 July 2015, a total of 260 and 192 leaf samples were collected for CMD and CBSD, respectively, from 107 fields across the country. Whitefly samples were collected where present.

CMD and CBSD incidence

Incidence is the proportion of plants that show symptoms of disease, and severity is the average of diseased plants assessed during the survey.

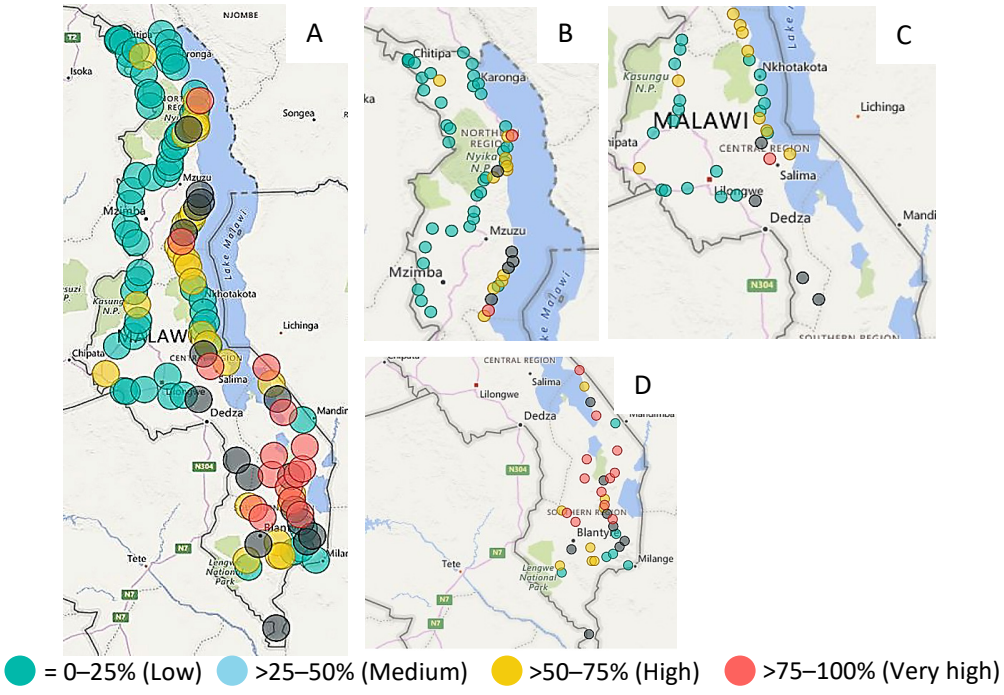

Figure 3 CMD incidence 2013 survey: (A) Overview of incidence at locations surveyed. Detailed incidence levels in Northern Malawi (B) Central Malawi (C) and Southern Malawi (D)

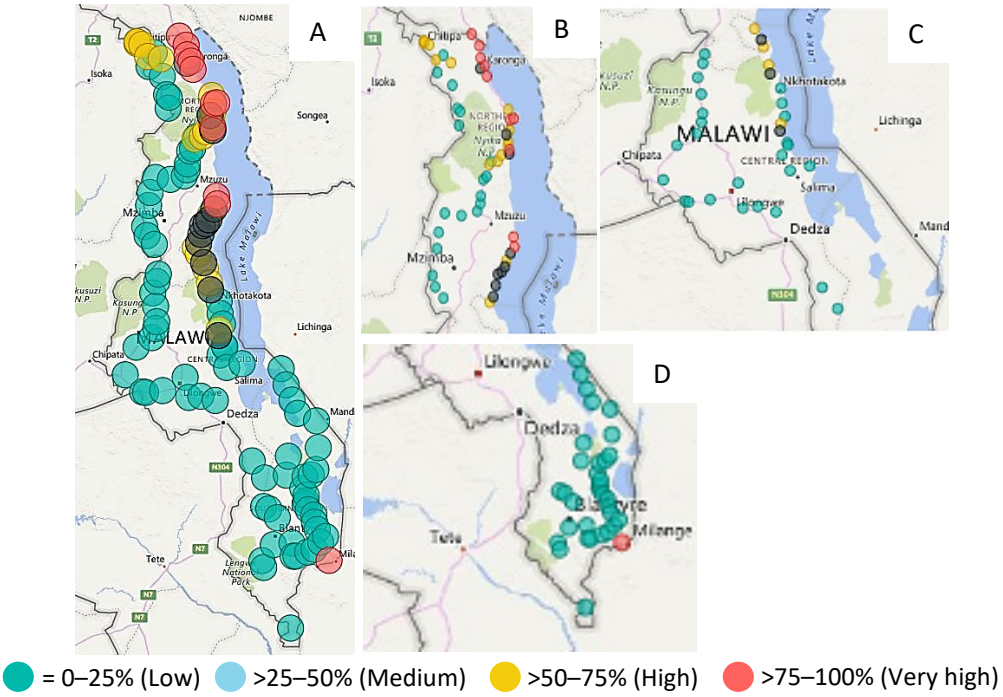

Figure 4 CBSD incidence 2013 survey: (A) Overview of incidence at locations surveyed. Detailed incidence levels in Northern Malawi (B) Central Malawi (C) and Southern Malawi (D)

In 2013, CMD was observed throughout all districts and in almost all fields sampled except for Mzimba district, but incidence varied considerably among those districts and regions (Figure 4 and Figure 5). Survey data showed that CMD incidence was generally low in the northern (19.2%) and central region (21.9%) and highest in the southern region (57.8%). Using the severity index range of 0–5 (where 5 indicates a high level of infection), mean CMD severity ranged within 2.2–2.8. Individual district scores showed that Nsanje (Southern region) had the highest score (3.9).

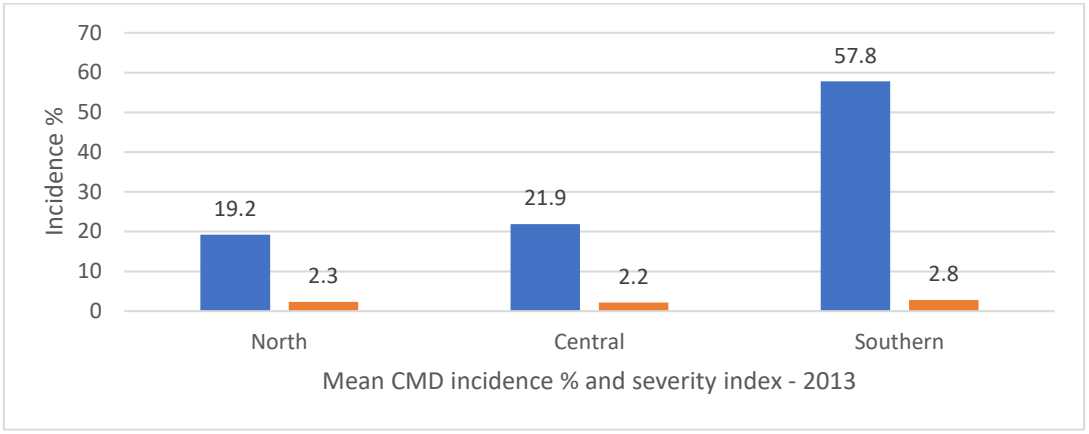

Figure 5 CMD incidence % and severity index – 2013 survey

Similarly, in the 2015 survey, CMD incidence was lowest in northern region and highest in the south (Figure 5 and Figure 7). The CMD incidence varied considerably among the districts surveyed. At individual district level, it was highest in Balaka in the south (100.0%) and lowest in Chitipa in the north (2.2%). This pattern, as also seen in 2013, suggests that Balaka could be one hotspot area for CMD. Individual district scores showed that 33% of districts in the south had a severity score exceeding 3.

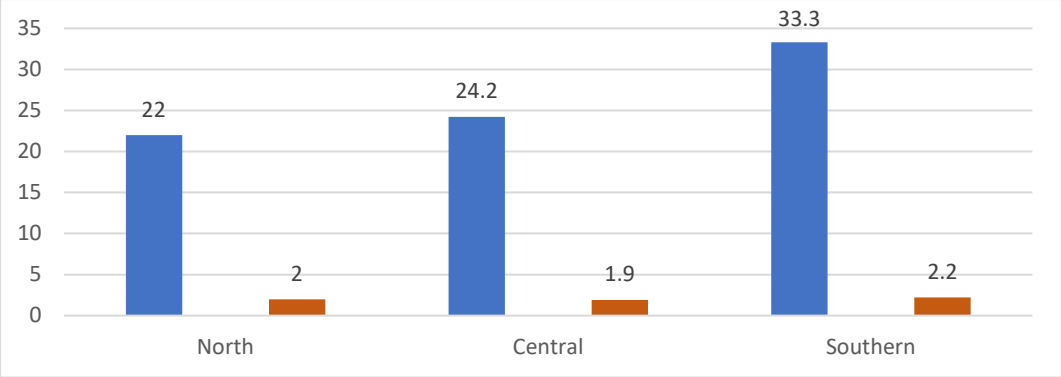

Figure 6 Mean CMD incidence % and severity index – 2015 survey

CBSD incidence and severity

The CBSD foliar incidence is the percentage of plants expressing CBSD symptoms in leaves. In the 2013 survey (Figure 7), there was high variation in CBSD incidence among the districts sampled, in the range of 0–79.3%. Highest CBSD incidence was in Karonga (79.3%), a district bordering Tanzania. The CBSD incidence was high in the northern (38%) and lowest in the southern region (5.9%). The average CBSD foliar severity was moderate in all districts surveyed, with a mean score of 2.4.

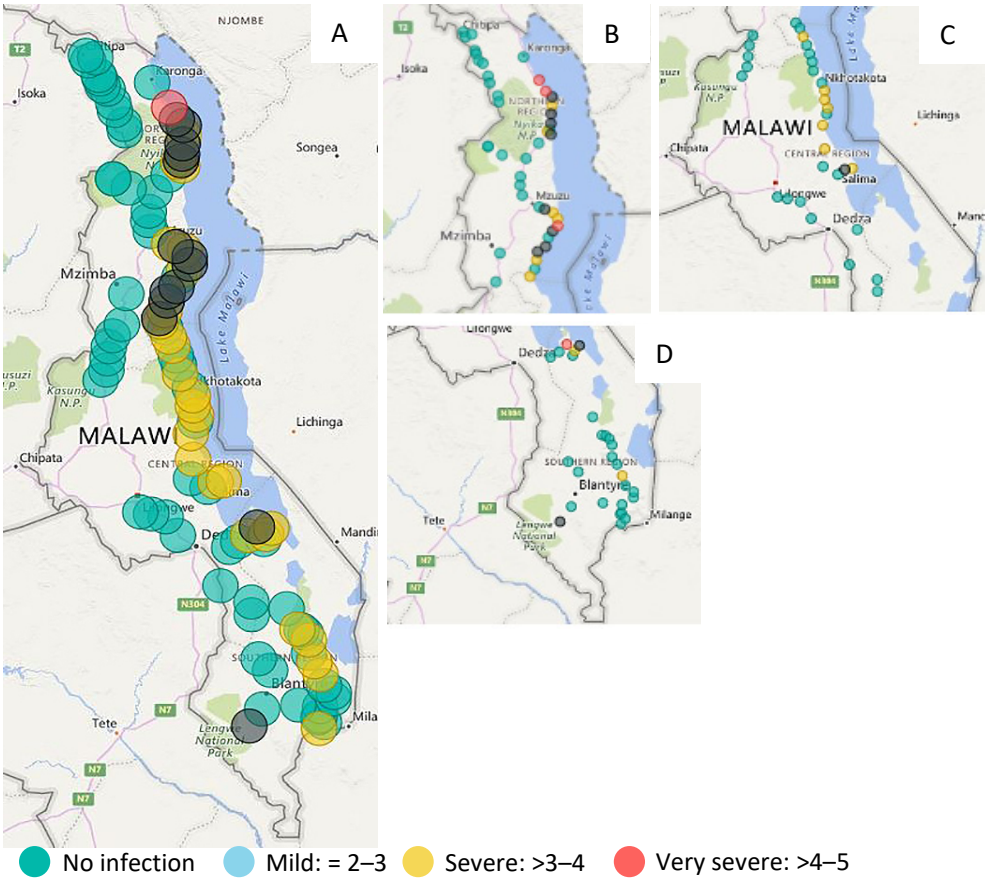

Figure 7 CBSD incidence 2015 survey: (A) Overview of incidence at locations surveyed. Detailed incidence levels in Northern Malawi (B) Central Malawi (C) and Southern Malawi (D)

In 2015, CBSD incidence varied among the districts surveyed, ranging within 1.3–70.7%. On average, the northern region had the highest (25.1%) CBSD incidence compared to the south (11.0%) and central (11.6%) regions. Similarly, the 2013 survey showed high CBSD occurrence in the northern region compared to the other two regions. Thus, the CBSD distribution had not changed much.

However, the CBSD incidence in the southern region had increased. Among the 21 districts sampled, CBSD incidence was highest in Salima district (70.7%) and lowest in Mulanje district (1.3%). The CBSD severity score varied considerably among the districts sampled: highest in Karonga (3.4) and lowest in Machinga (2.0) district.

Whitefly abundance

The mean number of adult whiteflies per plant was generally low in all districts surveyed in 2013, with an overall average of 0.7 whiteflies per plant. The highest number of adult whiteflies per plant was in Phalombe districts (5.1), followed by Chikwawa (3.2) and Mulanje (3.9) districts (Table 1). The low numbers of adult whitefly suggest that the CMD and CBSD incidence observed during the study were mainly due to use of infected planting materials and not to virus activity.

Table 1 Mean whitefly abundance in districts in the three regions surveyed in 2013 and 2015

Region	Fields	No. adult whiteflies/plant	Region	Fields	No. adult whiteflies/plant
	2013			**2015**	
North			**North**		
Chitipa	12	0.2	Chitipa	12	0
Karonga	10	1.9	Karonga	6	0.4
Rumphi	10	0.8	Rumphi	7	0.8
Nkhatabay	10	0.6	Nkhatabay	12	0.5
Mzimba	11	0.1	Mzimba	6	0
Central			**Central**		
Dedza	1	1.3	Dedza	2	0
Ntcheu	2	0.6	Ntcheu	3	1
Kasungu	8	0	Kasungu	10	0.3
Mchinji	3	1.3	Mchinji	1	0.2
Lilongwe	4	0.2	Lilongwe	3	0.2
Salima	4	0.3	Salima	6	1
Nkhotakota	10	0	Nkhotakota	13	0.4
Southern			**Southern**		
Blantyre	3	0.2	Blantyre	3	0
Chikwawa	2	3.2	Chikwawa	1	2.5
Chiradzulu	1	0	Chiradzulu	1	0
Mulanje	3	3.9	Mulanje	5	0.3
Phalombe	3	5.1	Phalombe	3	2.2
Zomba	6	0.3	Zomba	5	2.5
Machinga	5	0	Machinga	2	0
Mangochi	5	0.1	Mangochi	6	1.9
Balaka	2	0.2	Balaka	1	0

Similarly, in 2015, whitefly mean counts were very low in all districts surveyed, ranging from 0.3 to 2.5 adult whiteflies per plant, and an overall national mean of 0.6. The highest whitefly count was observed in Southern region.

Characterization of emerging viruses

Cassava virus isolates sequenced and analyzed

Plant materials from field surveys across Malawi were subjected to molecular tests using CMB and CBSV specific primers and were consequently sequenced using both the Sanger method and Illumina deep sequencing techniques.

A total of 26 sequences were generated for phylogenetic and evolutionary analyses using (BEAST) the Bayesian Evolutionary Analysis Sampling Trees software (Suchard et al, 2018) – see Figure 8. In addition to cassava viruses previously identified in Malawi – CBSV, UCBSV, *East African cassava mosaic Malawi virus* (EACMMV), *East African Cassava Mosaic Cameroon virus* (EACMCV) and *South African cassava mosaic virus* (SACMV) – our analyses revealed the presence of previously unreported Cassava virus C (Mtonga, 2018) and an Ampelovirus (Mbewe, unpublished) from Malawi. The data were not adequate enough to suggest that Cassava virus C isolate from Malawi was a species distinct from the Ivory Coast isolate. A separate finding was that computational analysis resulted in identifying a divergent CBSV subgrouping based on the *P1* gene (Mbewe et al., 2017a), as well as a genetically divergent UCBSV isolate (Mbewe et al., 2017b).

Cassava mosaic begomoviruses

Several begomovirus species and strains causing CMD have been reported in Malawi. In the 2013 survey, cassava leaf samples with conspicuous CMD symptoms were collected and subjected to PCR. A total of 259 DNA samples were analyzed for CMBs, of which 111 (43%) tested positive. EACMMV was detected in northern, central and southern regions of Malawi, while SACMV was only detected in southern region. No EACMCV was detected in the 259 samples analyzed. A total of 16 (6.4%) samples tested positive to dual infections. The common dual infection was EACMMV + SACMV (15), and only one sample tested positive to EACMMV + EACMCV and none to EACMCV + SACMV. Of all districts surveyed, Nkhatabay had the highest occurrence of EACMMV (eight), followed by Mzimba (seven). Fourteen samples were positive to universal EAB555F/EAB555R (Ndunguru et al., 2005) but did not amplify any of the specific primers. It is possible that these belonged to *East Africa cassava mosaic Zanzibar virus* and *East Africa cassava mosaic Kenya virus*.

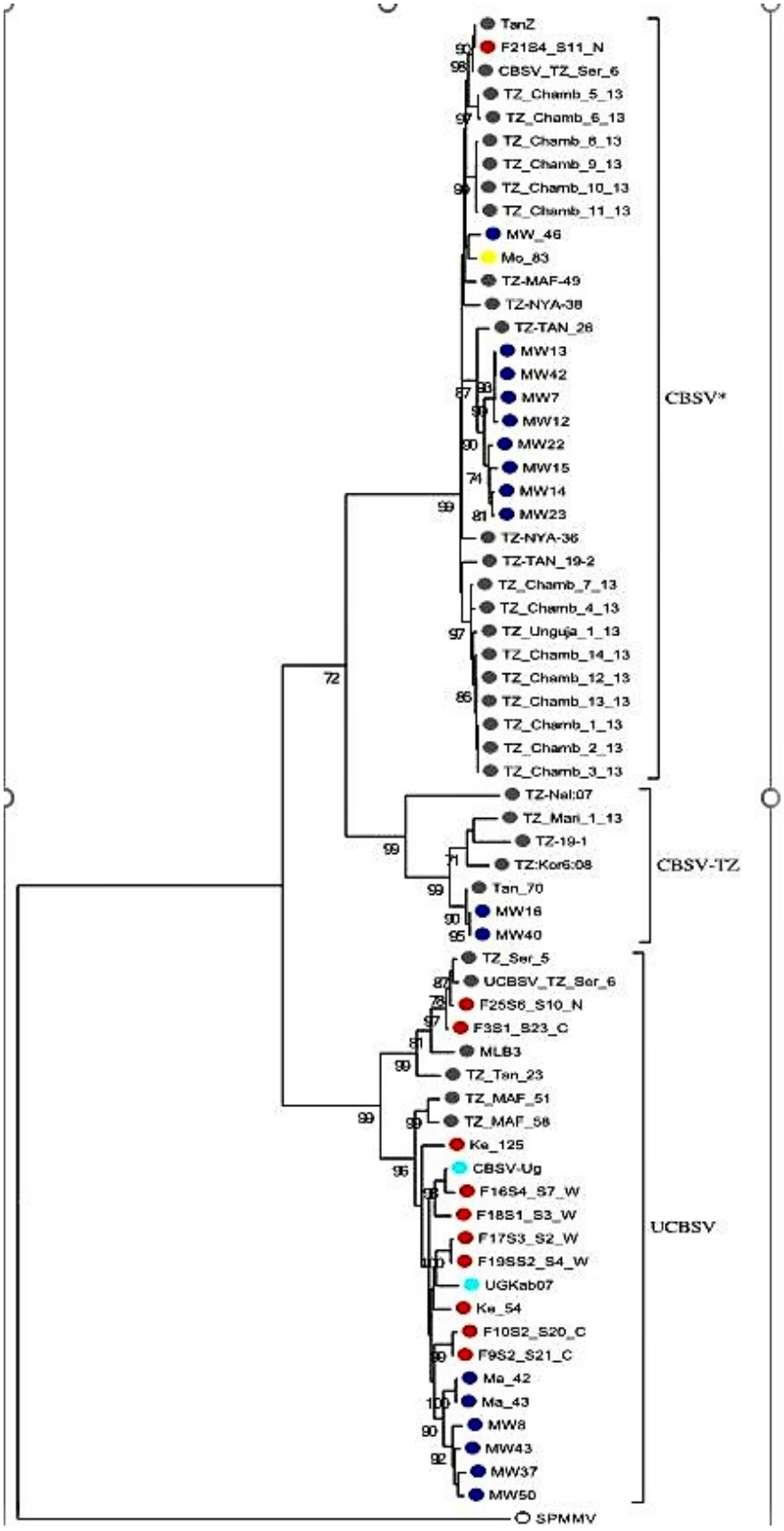

Figure 8 Maximum likelihood tree of partial *P1* gene sequences of CBSV isolates (Mbewe et al., 2017a)

In the 2015 survey, out of 257 samples analyzed using PCR, 65 (25.3%) were positive for cassava mosaic begomoviruses (CMB). The EACMMV was the most common CMB in Malawi with 45 (69.23%) samples positive for EACMMV, and only one sample tested positive to EACMCV (Figure 9). There was co-infection of EACMMV + EACMCV in 15 samples representing (23.1 %), and EACMMV + SACMV in one sample (0.4%). Three samples (4.61%) had all three viruses: EACMMV + EACMCV + SACMV.

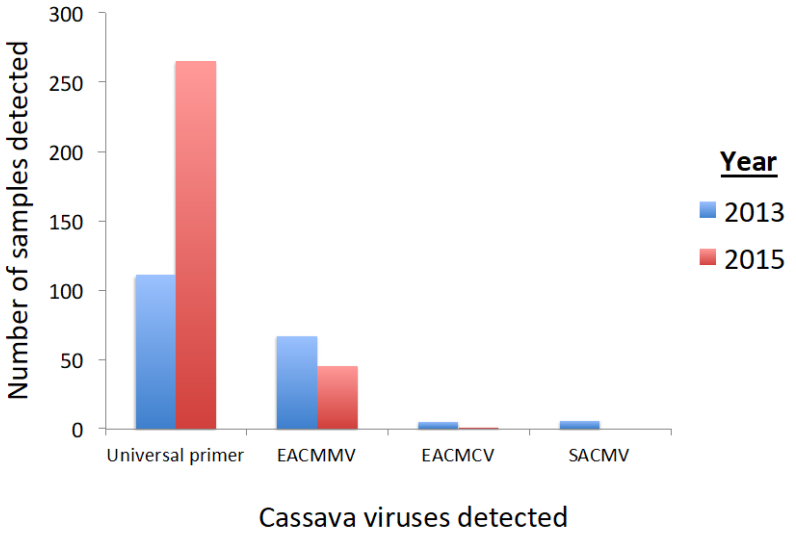

Figure 9 Detection of cassava mosaic begomoviruses in Malawi

Ninety-four RNA samples were analyzed, of which 29 (31.9%) gave positive results. Among these, 11 (37.93%) had single infection of CBSV, 15 (51.72%) had UCBSV alone and three (10.34%) had dual infection of CBSV + UCBSV. Single UCBSV infection was prevalent in Nkhatabay (10), while single CBSV was most frequent in Rumphi (five) and Karonga (four). The CBSV results suggest that UCBSV is the most common species in Malawi.

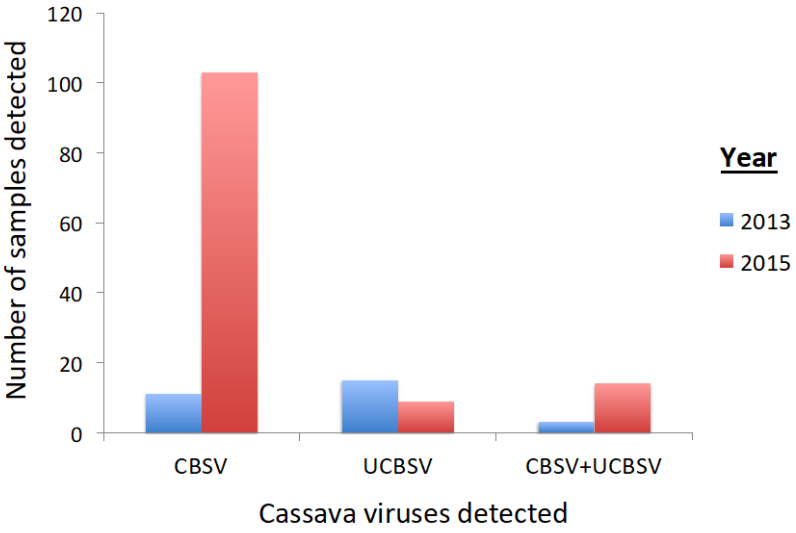

Figure 10 Detection of cassava brown streak ipomoviruses in Malawi

In the 2015 survey, a total of 192 cassava leaf samples were analyzed for CBSVs, of which 58.3% tested positive to CBSV (103) and UCBSV (nine), suggesting that CBSV was the most common species in Malawi. This differed from the 2013 survey, in which UCBSV was common (Figure 10). CBSV occurred in all three regions of Malawi, while UCBSV was detected in the northern and central regions but not in the southern region. Prevalence of CBSV was high in Nkhotakota (18) and Nkhatabay (16), and UCBSV was high in Nkhatabay (4) district. A total of 14 samples (12.61%) had dual infection of CBSV + UCBSV. All samples collected from Chitipa and Mzimba were free of CBSVs.

Changes in cassava virus disease incidence 2013–2015 surveys

- Mzimba was CMD-free in 2013 but CMD was present in 2015
- There was no SACMV in the northern region in 2013 but it was detected in 2015
- Balaka had the highest CMD incidence in both surveys
- Southern region had the highest CMD incidence in both surveys
- Northern region had the highest CBSD occurrence.

In 2013, UCBSV was high; however, in 2015, CBSV were much more common than UCBSV. CBSD was distributed more along the lakeshore, but laboratory results showed higher CBSV incidence in the south than in previous surveys. This calls for strategies to slow or stop the spread of CBSD in the country. The CMD incidence was high along the lakeshore and in the southern region compared to the upland areas of central and northern region. These areas of low incidence for both CMD and CBSD would be suitable for establishment of cassava planting materials.

Alternative hosts for CBSVs and CMBs and associated insect vectors identified

Survey of neighboring uncultivated plants

During the survey, leaf samples of wild plants with virus-like symptoms were collected for virus testing in the laboratory with the aim of identifying the potential reservoir host for viruses in Malawi. Seven plant species showing CBSD-like (brown streaks) and CMD-like (green or yellow mosaic) symptoms were collected during the survey for virus testing (Table 2). These plants were tested for presence of CBSVs and CMBs and all were found to be free from both viruses and were not found of immediate concern to the cultivation of cassava in the areas surveyed. All samples tested negative for both CMD and CBSD in the laboratory analysis.

Although all the wild samples tested negative, this does not mean they were clean of virus and this can be caused by the specificity of primers used. Deep sequencing techniques could reveal possible viral presence and establish evolutionary relationships between possible novel viruses and those from cassava fields. This is an area to be considered in the future.

Table 2 Symptomatic uncultivated plants tested for CMD and CBSD

Uncultivated plants	District	Symptoms	Result of test
Pumpkin (*Curcubita* spp.)	Balaka	CMD-like	Negative
Fig tree (*Ficus carica*)	Nkhatabay	CBSD-like	Negative
Avocado (*Persea americana*)	Nkhatabay	CBSD-like	Negative
Pawpaw (*Carica papaya*)	Mangochi	CMD-like	Negative
Lemon tree (*Citrus limon*)	Mangochi	CBSD-like	Negative
Pawpaw (*Carica papaya*)	Rumphi	CMD/CBSD-like	Negative
West Indian lantana (*Lantana camara*)	Nkhatabay	CMD-like	Negative

Characterization of disease vectors

Whitefly characterization

To identify potential insect vectors of cassava viral diseases, whitefly and other insect samples were collected during the surveys. A total of 67 whitefly samples were collected for characterization during the 2013 and 2015 surveys. Project staff were trained on whitefly DNA extraction and on the detection of viruses from the extracted DNA. Dr Sseruwagi and Ms Leonia Mlaki from the Tanzania Agricultural Research Institute (TARI)–Mikocheni, Tanzania, facilitated the training. Preliminary results indicated the occurrence of *B. tabaci* SSA1 species with two genetic groups: Subgroup 2 and Subgroup 3.

SECTION TWO: Integrated pest management

Supporting clean seed systems for farmers

Conventional breeding support

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

The human capacity on cassava viral diagnostics that was developed under the CDP has benefited other projects that were working toward the availability of virus-free planting materials for cassava.

Breeders' seed and multiplication plots were monitored for CMD and CBSD, and leaf samples were collected for virus indexing. A total of 4248 cassava leaf samples were collected and screened for cassava viruses (Table 3) from regional multiplication trials under the Cassava Varieties and Clean Seed to Combat CBSD and CMD Project (5CP) project from five sites in Malawi: Makoka, Chitedze, Chitala, Mkondezi and Vinthukutu.

Table 3 Samples indexed for other projects

Project	Disease screened	Number of samples analyzed	Virus free	Virus infected
ASWAP-SP initiative[1]	CMD & CBSD	1500	1066	434
IITA[2]-GIZ[3] funded project on cassava commercialization[2]	CMD & CBSD	1500	1454	46
AGRA-funded project on breeder and foundation seed multiplication[4]	CMD & CBSD	1248	711	537
Total		**4248**	**3231**	**1017**

[1] Agriculture Sector-wide approach support program (ASWAP-SP); [2] International Institute of Tropical Agriculture (IITA), [3] Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ); [4]Alliance for Green Revolution in Africa (AGRA)

Reaching farmers directly and through partners

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

Baseline survey on farmers' knowledge on cassava viral diseases

The objective of the survey was to obtain information on farmers' knowledge on cassava viral diseases, farmer cultivation practices and how they influence the spread of viral diseases (Figure 11). Farmers were interviewed on their years of experience in cassava cultivation, constraints in cassava cultivation, preferred varieties and access to extension services.

Farmers interviewed mentioned Sauti, 20:20, Kolobeka, Matako Lembwende and Nyantonga as their preferred varieties due to being early maturing, high yielding and disease resistant. These surveys indicated that there was a need for improved, disease-free cassava planting materials and training in better farming methods and management of cassava virus diseases.

Figure 11 Project staff doing national disease survey in 2015

In 2016, farmers who participated in the demonstration plots were trained on the identification and management of cassava pests and diseases, clean cassava seed production and good agronomic practices for cassava cultivation. The training was conducted during 2–5 October 2016 when the plants were eight months old, and was attended by 25 farmers (12 males and 13 females) and three male extension staff.

Training of extension workers on cassava disease identification and management

In line with this objective, two-day training events were organized in 2015 in each of the three regions of Malawi. Two training sessions were organized jointly with the project coordinator for the ASWAP project. A total of 73 participants including extension staff and research technicians were trained (Table 4).

Table 4 Summary of training for extension staff and research technicians

Region	Females	Males	Total
North	4	22	26
Central	11	23	34
South	5	8	13
Total	**20**	**53**	**73**

To ensure that all participants shared a common understanding of the information disseminated, the training focused on harmonizing messages on viral disease identification, and on the management and implementation of trials.

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

The objective was to demonstrate the benefits of using improved and indexed cassava planting materials to farmers. The demonstrations were designed so that the demonstration plots would be hosted by a group of farmers, and that the planting would comprise two cassava varieties: an improved variety identified through research and one farmer-preferred local variety. However, in the first year, it was not possible to organize farmers into groups, and thus demonstrations were hosted by lead farmers. Farmers from the surrounding communities were invited on specific occasions for training on CMD and CBSD symptom recognition and management strategies.

Three demonstration plots were set up in each of the 2014/15 and 2015/16 seasons: two in Vinthukutu and one at Nathenje using indexed planting materials along with one local variety. At both sites the indexed varieties used were Sauti, Sagonja, Kalawe and Mpale. The following local varieties were used: Mbundumali at Nathenje; and Matakolembwende and Nyautonga at Vinthukutu. In December 2015, the demonstration field at Nathenje was ratooned and planting materials were distributed to farmers for further multiplication. The plot was maintained, and materials harvested in December 2016, and planting materials were shared to 25 farmers within the Nathenje area.

The 2014/15 demonstration plots in Karonga were not harvested as they were damaged by livestock. In the 2015/16 season, two demonstration plots were established in the village of Mng'ombwa in the Hara section of Vinthukutu Extension Planning Area (EPA). Farmers were given virus-free planting material from varieties Sagonja, Sauti, Mpale, Kalawe and Mbundumali to plant in the demonstration plots along with their chosen local variety. Farmers near the demonstration plots were trained in cassava disease and pest identification and management.

During the 2016/17 season, two new farmer groups were formed and given clean planting materials. Each demonstration group (Mwawi and Kambwera at Vinthukutu EPA in Karonga district) had a plot of 10 ridges by 10 m where one improved variety (Sagonja) and a local variety (Matakolembwende) were planted. At each demonstration plot, a bulk crop of Sagonja variety (approximately one acre) was multiplied.

Planting materials from the demonstrations and seed multiplication were distributed to farmers. A total of 52 farmers benefited from the clean planting materials harvested from plots at Vinthukutu and Nathenje (Table 5).

Table 5 Cuttings distribution and farmer demographics

Region	Number of cuttings shared	Female	Male	Total
Northern	9800	2	5	7
Central	20,600	15	30	45
Total	**30,400**	**17**	**35**	**52**

Development and dissemination of information materials

Disease awareness

To facilitate message dissemination on cassava viral diseases, a media tour was organized during 21–23 June 2015 – a total of 12 journalists from seven media houses participated. The tour started with formal training on causes, symptoms and management of both CMD and CBSD, followed by a field visit to cassava fields for a practical session on disease recognition (Figure 12).

Figure 12 Journalists training on cassava viral diseases, Salima, June 2015: (left) participants during formal training in class, (right) participants during practical field session

Apart from the media tour, journalists also participated in the CDP Fifth Annual Meeting held at the Golden Peacock Hotel, Malawi, during 11–13 May 2015. The Minister of Agriculture Dr A. Chiyembekeza officially opened the meeting. After this meeting two articles were published – in print and electronically (MBC, 2015; The Nation, 2015).

Participation in agricultural shows and exhibitions

The project team participated in one national agricultural fair held in Blantyre during 28–30 August 2014. During the fair, the team displayed samples of clean and diseased cassava planting materials, posters on the incidence and severity of cassava virus diseases in Malawi in 2013 and different cassava varieties. The President of the Republic of Malawi and Chief Justice were the high-profile guests at the Department of Agricultural Research Services (DARS) pavilion (Figure 13).

Figure 13 National Agricultural Fair at Trade Fair Grounds, Blantyre, August 2014. (A) Department of Agricultural Research Services pavilion, (B) print materials on display, (C & D) Director of Research briefing the President of The Republic of Malawi, HE Prof Arthur Peter Mutharika

Field days participation

The team participated in two field days. On 25 April 2016, the team participated in a field day organized by Lilongwe Agricultural Development Division. The field day took place at Nathenje EPA where a demonstration had been established under this project (Figure 14). The carry-home message was use of improved varieties and clean planting materials. The field day was attended by 178 farmers: 70 male, 86 female and 22 children.

Figure 14 Farmers participating in the Nathenje Field Day 2016

On 31 March 2017, the team participated in field day organized by Chitedze Research Station (Figure 15). The message centered on use of clean planting materials, and CMD and CBSD recognition and management. Some of the guests who visited our pavilion were farmers, students,

chiefs and community leaders. The guest of honor was the Deputy Minister of Agriculture, Mr. Aggrey Massi, accompanied by some members of the Parliamentary Committee on Agriculture.

Figure 15 Farmers participating in the Nathenje Field Day 2016

Official visitors

Four members of parliament from the Republic of Ireland visited Malawi in July 2015 (Figure 16). They visited the Chitedze Biotechnology Laboratory where they were briefed by the Country Team Leader on activities being carried out and the future plans for the laboratory. The purpose of the visit was to get an appreciation of what a sister project (funded by Irish Aid) was doing to address food security in Malawi. This project made use of the CDP-funded biotechnology laboratory.

Figure 16 Members of Parliament from Ireland visiting Chitedze Biotechnology Laboratory, July 2015

Building sustainable regional capacity

Strengthening stakeholder linkages

Stakeholders engagement

Meetings were conducted annually with the CDP team from Tanzania Agriculture Research Institute (TARI)–Mikocheni. Discussions during these visits enabled the Malawi team to carry out their activities and meet their objectives.

Table 6 Partners and stakeholders visited during the impact assessment baseline study and monitoring and evaluation missions for the 'Disease diagnostics for sustainable cassava productivity in Africa' project, 2014

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
Jomo Kenyatta University of Agriculture and Technology (JKUAT)	Thika Road, Nairobi	University (Project partner)	Dr Elijah Ateka (Project Country Team Leader), Samuel Mwaura (Research Assistant), Timothy Makori (Technician)	Project management, training, supervision of students and research	Dr Elijah Ateka and Mr. Samuel Mwaura
Department of Agricultural Research Services, Chitedze Agricultural Research Station	Lilongwe	NARS (Project Partners)	Dr Ibrahim Benesi (Team Leader), Mr Albert Mhone (Assistant Team Leader), Ms Ruth Semu Mwase (Research Assistant), Mr Michael Benjala (Research Assistant)	Hosting the project, breeding for resistance to CMD and CBSD and disease diagnostics	Dr Ibrahim Benesi, Mr Albert Mhone, Ms Ruth Mwase, Mr Michael Benjala
Department of Agricultural Research Services, Chitedze Agricultural Research Station	Lilongwe	NARS (Non-Project Staff)	Mr Elisa Mazuma (Plant Pathologist and Deputy Director Plant Protection), Mr David Kamangira (Senior Deputy Director of Technology Management and Reg Services), Ms Clementina Banda (Ass Agric Research Officer), Mr Harry Mleta (Agric Research Officer), Mr John Mphaya (Agric Research Officer)	Research and Improvement of cassava	Mr Elisa Mazuma, Mr David Kamangira

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
Department of Agricultural Research Services, Chitedze Agricultural Research Station	Lilongwe	NARS	Ms Sarah Chilungo (Breeder), Mr Wilson Chifudsa (Head of Seed Quality Control Unit)	Cassava breeding for improvement and resistance to CMD and CBSD and Seed Certification	Ms Sarah Chilungo, Mr Wilson Chifudsa
University of Malawi	Lilongwe	Collaborator	Dr Alfred Maluwa (Chairman of National Working Group of Sweet Potato Innovation Platform)	Research and Improvement of cassava	Dr Alfred Maluwa
Lilongwe University of Agriculture and Natural Resources, Department of Crop and Soil Sciences	Lilongwe	University	Dr Moses Maliro (Lecturer in Plant Breeding)	Training and Supervision of Students	Dr Moses Maliro
International Institute of Tropical Agriculture (IITA), Malawi	Lilongwe	BMGF supported project on cassava breeding - New Cassava Varieties and Clean Seed to Combat CBSD and CMD (5CP)	Mr Hastings Musopole	Cassava Breeding and Improvement	Mr Hastings Musopole
International Institute of Tropical Agriculture (IITA), Malawi	Lilongwe	Collaborator	Mr Christopher Moyo (Research Associate – Cassava Breeding) Regional Cassava Breeding Project	Cassava Breeding and Improvement	Mr Christopher Moyo
International Potato Centre (CIP)	Lilongwe	Collaborator/ Private Seed Producer	Dr Philip Demo (Country Representative)	Cassava Breeding and Seed multiplication and distribution	Dr Philip Demo
Ministry of Agriculture and Food Security, Department of Extension Services	Lilongwe	Government extension	Dr Clodina Chowa (Deputy Director Extension Methodology and systems)	Training of farmers and cassava seed multiplication and distribution	Dr Clodina Chowa
Ministry of Agriculture and Food Security, Department of Extension Services	Chilumba Extension Planning Area, Karonga	Government extension	Mr Dingani Zalilo (Extensionist), Mr Jeremiah Moyo (Extensionist)	Training of farmers and cassava seed multiplication and distribution	Mr Dingani Zalilo

Institution	Location	Partnership/ Type of stakeholder	Respondent(s)	Role	Contact person(s)
Farmers	Chambogho, Karonga	Farmer	Ms Teleza Zgambo, Ms Febbie Mzembe, Mr Elijah Mwafulirwa, Mr Wachepa Mwafulirwa and Mr Vasco Msiska	Cassava production	Mr Elijah Mwafulirwa

Collaborations

The facilities and expertise acquired as a result of CDP enabled the Malawi team to work with external organizations and educational institutions (Table 7).

Table 7 Collaboration with external organizations

Institution	Nature of collaboration	Contact person
Bvumbwe Research Station	Genotyping of breeders' seed for regional trial (5CP)	Dr Obed Mwenye
Lilongwe University of Agriculture and Natural Resources (LUANAR)	• BSc Students carrying out their practicals on CMD and CBSD • MSc student carrying out their research using the CDP facility	Dr Abel Sefasi Misheck Bushiri
International Institute of Tropical Agriculture (IITA)	CMD and CBSD diagnostics for IITA-GIZ cassava multiplication fields	Dr Pheneas Ntawarunga, Chris Moyo
Agricultural extension Services (Karonga ADD-Vinthukutu EPA)	Cassava seed multiplication and demonstrations Training of trainers	Extension officer, Vinthukutu EPA Extension Officer, Nathenje Training Centre
University of Free State, South Africa	Masters student, registered with University of the Free State, used CDP facilities toward his Master's thesis	Hastings Musopole

Strengthening human capacity and infrastructure

The CDP has greatly enhanced Malawi's human resource capacity (Table 8). Training undertaken by project personnel includes the molecular detection of cassava viruses, financial management and bioinformatics. A total of 13 officers have been trained. Additionally, the CDP project funded one MSc and one PhD student at Rutgers University, USA, and Makerere University, Uganda.

Table 8 Training courses undertaken by project staff during Phase II of CDP

Dates	Course title	Venue	Staff
May 2013	Financial Management and Accounting package	Dar es Salaam, Tanzania	Dr Benesi, Neston Chimosola and Joel Kwelepeta
22 September to 5 October 2013	Molecular detection of plant viruses	Dar es Salaam	Albert Mhone and David Mbalangwe
July 2014	Leadership Training for institutional Directors	Kigali	Dr Makumba
August 2014	Molecular detection of plant viruses	Dar es Salaam	Sarah Chilungo and Michael Benjala
26–31 October 2014	Intellectual Property Rights	Nairobi, Kenya	Albert Mhone
2014	PhD Research work	DSMZ Plant Virus Department, Germany	Willard Mbewe
2014–2017	MSc	Makerere University	Andrew Mtonga
22–24 February 2016	Bioinformatics	Dar es Salaam	Andrew Mtonga and Albert Mhone
11–13 April 2016	Financial Management Essentials	Dar es Salaam	Albert Mhone and Lucy Chapweteka
June 2016	AgShare Training	Lusaka, Zambia	Willard Mbewe and Andrew Mtonga
December 2016	Data Entry and Assembly Workshop	Dar es Salaam	Ruth Semu and Ruth Kaunda
19–22 January 2017	Leadership Training for Scientists	San Diego, California, USA	Willard Mbewe and Andrew Mtonga
March–April 2017	Analysis of siRNA sequencing data for characterization and discovery of viruses	CIAT, Colombia	Andrew Mtonga

Apart from providing the project staff and government workers with the tools and environment required for research, the CDP project support to the Chitedze Biotechnology Laboratory has benefited several stakeholders, especially students from colleges and universities. During the project period, LUANAR has sent students (Diploma, BSc and MSc students) for familiarization with equipment and procedures used in a biotechnology laboratory. A total of 176 students had access to the laboratory (Table 9). In addition to these short visits, biotech students from LUANAR have been attached to the laboratory as part of their course requirement. During the students' attachment, detection of viral diseases in cassava was one of the major practical activities they undertook.

Table 9 Student visitors to the Chitedze Biotechnology Laboratory

Student category	Areas of specialization	Numbers
Masters	Horticulture, plant breeding, virology	13
Bachelors	Crop sciences, agriculture, biotechnology	143
Diploma	Agriculture	20
Total		**176**

Infrastructure strengthening

The DARS benefited from several infrastructural support items in Phase II of the CDP. These include screenhouse renovation, project vehicle and laboratory equipment.

Access to water

The laboratory has been operational since 2014 but without running water. Water for washing glassware and for other laboratory uses was collected from other offices and hand delivered. In 2017, a 5000-L tank was erected and connected to the laboratory courtesy of CDP.

Renovation of screenhouse

One screenhouse was renovated in 2013 and early 2014 under this project to complement efforts from the disease diagnostics laboratory for the provision of clean cassava planting materials. Cassava plants that have been tested as virus-free from the laboratory were planted in tubes and kept in the screenhouse to protect them from virus disease infection caused by whiteflies.

Laboratory equipment

A number of laboratory equipment items were purchased within the project period. These include gel documentation system, double water distiller, analytical balance, micropipettes, deep freezer and upright fridge (Table 10). The project also supported the procurement of laboratory consumables.

Table 10 Equipment purchased by DARS 2013–2017

Asset	Serial no.	Date of purchase	Current location	Status
Deep freezer	KCG 570/7	2017	Chitedze Biotech Lab	Working order
Upright fridge	BHSTDJ 1642582	2017	Chitedze Biotech Lab	Working order
Water distiller	FI-0020034	2015	Chitedze Biotech Lab	Working order
Gel documentation system	A 113554	2015	Chitedze Biotech Lab	Working order
Analytical balance	ME 204	2015	Chitedze Biotech Lab	Working order
Nissan Patrol vehicle (BS 4159)	Chassis No.: JN1TCSY61Z058515; Engine: TD42225522	2013	Chitedze	Working order

SECTION THREE: Impacts, success stories and learning outcomes

Impact of CDP in Malawi

Impact area	Impact
How many students were trained by this project directly and indirectly	A total of five students were trained: • Three MSc • Two PhD.
No. of projects using the CDP facilities	Three projects: • IITA-GIZ • ASWAP • AGRA.
No. of students and/or staff using facilities and reagents of CDP	Used by 176 students and staff to carry out their experiments.
No. of people that have been inspired by the project	• Five scientists submitted their research proposals following success stories learned from CDP • Several hundred farmers realized the benefits of obtaining clean seed.
Institutional visibility – recognition of the institute's capacities	• Before the CDP intervention, the molecular biotechnology laboratory did not exist. Since laboratory establishment, LUANAR has been sending students to it • DARS was present at the national agricultural show where it showcased the work done in the laboratory and the new varieties of cassava.
Infrastructural capacity – helping student execute their project	• Well-equipped molecular biology genotyping equipment • Well-functioning tissue culture laboratory and growth room • Three well-equipped Biosafety Level 2 laboratories • Well-equipped molecular diagnostic laboratory • Two screenhouses for insect-controlled experiments • Fast internet facilities • Accessibility to peer-reviewed journals through AGORA, Plant Disease, Phytopathology, Molecular Plant-Microbe Interactions journal subscriptions in 2014–2015 • Bioinformatics facility for sequencing analysis.
New stakeholders interacting with the project	• A number of stakeholders have interacted with the project, including LUANAR.

Service the lab has provided	• A total of 4258 cassava samples were cleaned and indexed from other projects • The laboratory provided research services to MSc and PhD students (for the number of students trained see Table 9).
How many farmers have benefited either directly or indirectly	Training and materials benefited 255 farmers: • CDP trained farmers in good cassava agronomic practices, pest and disease identification and management practices.
New collaborations/collaborative projects	Two new collaborations: • IITA-GIZ on cassava commercialization • ASWAP on the characterization on viral diseases in banana, sweet potato and cassava.
People using information generated by this project	• Extension circulars were provided to farmers and are being used on disease diagnosis and field management • Other projects (Table 7) are using clean seed materials; awareness of these was promoted by CDP.
Benefits to the government – extension training, inspectors and regulators	• Government extension workers benefited from a number of trainings and seminars on field CBSD and CMD diagnostics • Seed inspectors were also involved in seed systems and field identification of pests and diseases.
Advocacy – impacts on policy, etc.	• CDP contributed knowledge that was used in incorporating cassava into Malawi's new National Seed Policy. Previously the seed policy neglected vegetatively propagated crops.
Publications and other communications including other communication materials	• Three scientific papers in peer-reviewed journals • Four conference papers • Three newspaper articles • Two online news stories (see References).
Increase in crop yield and incomes (especially farmers who used clean materials)	• Farmers attributed high yields due to project intervention. The use of clean planting materials, proper phytosanitary measures etc. contributed to the high yields • Some farmers have started cutting cassava as seed. This is a booming business among smallholder farmers where those with large pieces of land and irrigation facilities can make more than MK10 million (US$13,333.00) per season.
Meetings and conferences attended – for the whole team	• Rutgers University Microbiology Symposium, 6–9 February 2017, New Brunswick, New Jersey, USA

	• American Society of Microbiology Microbe Symposium, 1–5 June 2017, New Orleans, Louisiana, USA • International Plant and Animal Genome Conference (PAG XXV), 14–18 January 2017, San Diego, California, USA • 9th Virus Evolution Workshop, 9–11 March 2017, Pennsylvania State University, PA, USA • World Congress on Root and Tuber Crops. January 2016. Nanning, China.
New businesses initiated as a result of this project	• A number of businesses were motivated by the CDP project. The most notable include the seed system business (detailed above), where farmers can make more than US$13,333.00 per season, and the baking industry. Due to high yields, availability of cassava is enabling women to make cassava flour and to produce baked items such as cakes.
Involvement of vulnerable groups	• The project has involved women from the onset. They are among the most vulnerable groups in Malawi. The project has empowered them through a number of businesses as discussed above.
Change of farmers perceptions	A number of perceptions have changed due to CDP intervention. Notable ones include: • Some farmers thinking that CMD is not a disease but rather a nutritional deficiency • The use of clean planting materials as a tool for high yield. Farmers thought that it did not matter what planting material was used, since cassava can withstand 'harsh' environments • Farmers never imagined that cassava seed production could be a viable business. People are used to going to shops to buy well-packed maize seed but never thought cassava planting materials could also be a marketable business.
Other universities requesting to use the facilities/equipment	• LUANAR.
Minimizing/arresting brain drain	• Two postgraduate students and four project staff were employed by the project to work and be associated with project activities in Malawi. This contributed to minimize brain drain in the country.
Building a network of scientists	• The project has collaborated with scientists from other CDP partners in Africa (TARI, JKUAT, RAB, NaCRRI, ZARI and IIAM). Additionally, Rutgers

	University (USA), DSMZ Plant Virus Department (Germany), Makerere University (Uganda), North Carolina State University (NCSU, USA), Department of Agriculture and Food Western Australia and the University of Western Australia (UWA) and Virology Laboratory, CIAT (Colombia).

Success stories

CDP's work in Malawi

The CDP has been operating in Malawi since 2009 based at Chitedze Agricultural Research Station which is part of the DARS in Lilongwe. The project comprised core CDP team members, postgraduate students and government employees who provided laboratory and field support.

Figure 17 Farmers at one of their demonstration plots

Until recently, the only means of deciding if planting material was disease-free was through visual assessment – the lack of visible symptoms signified a disease-free plant. Through this type of assessment, plants have been distributed from one area to another in good faith, only to develop infection at a later stage of growth. This has contributed to the spread of CMD and CBSD throughout the country. The work carried out by the CDP team has contributed to the knowledge and yield of cassava in a number of ways, with the aim of combating the spread of CMD and CBSD.

Education of local farming communities

Early surveys of farmer knowledge about cassava diseases indicated a need for an education program to help farmers recognize the symptoms of viral diseases affecting their crops, and to provide them with the means to access disease-free material. The CDP project organized field days with an emphasis on disease symptom recognition and the use of clean planting material. During one of these events, some 180 visitors (male, female and children) were recorded, reflecting the interest of the farming community in improving their crops. The participation of the project team in agricultural fairs has also been an effective educational vehicle. Posters and plant samples

underlined the benefits of clean planting material. The presence of eminent visitors, such as the President of Malawi and the Chief Justice, helped to disseminate the message at the highest levels.

Detection of cassava viruses and production of clean planting materials

Through the CDP team's research, diagnostics tools have been used to detect the presence of viral diseases at molecular level. This work has enabled researchers to link disease symptoms to specific viruses. This important research step has provided the ability to identify those cassava varieties that are not affected by the known cassava viruses.

These disease-free varieties have been tested at the research facility and proven in the field through the demonstration-plot experiments carried out with the participation of farmers across Malawi. This has been a direct involvement with farmers who have been able to compare the yield of the tested varieties with their own preferred plants. The harvested cassava crop from these demonstration plots was shared among farmers, giving the latter a stock of clean planting material for their next planting phase.

Figure 18 Farmers harvesting cassava at demonstration plot in Karonga 2016 and showing the crop obtained

High yield from improved agronomic practices, pest and disease identification and management

Karonga is a traditional cassava growing area and cassava is one of their main staple foods. Mr Kanyimbo who lives in Vithukutu is one of the lead farmers who hosted the CDP cassava demonstration plot in 2015/16 growing season.

Figure 19 Vithukutu farmer, Mr Kanyimbo, displaying a good harvested of cassava from improved variety disseminated through demonstration fields

Mr Kanyimbo stated that he had been growing local varieties (Matakolembwende and Maso azungu), which were low yielding and prone to diseases. Additionally, they had been using ridges – which were very large and widely spaced – and this contributed to low yields.

Through the CDP, Mr Kanyimbo was trained in good cassava agronomic practices, pest and diseases identification and management practices and he also hosted a demonstration plot. Through that, he was introduced to new varieties of Sagonja, Kalawe and Mpale. The new varieties were tolerant to diseases and high yielding and the kondowole (fermented flour) processed from these varieties is acceptable (nsima is very white and cooked leaves are tasty) which has made his household more food secure.

After training, he adjusted ridge size and spacing, resulting in higher yields. He gave an example that he harvested 3.5 bags of 50 kg of Sagonja compared to 1.5 bags of Matakolembwende from the same area (one ridge of 10 m long). Through the training, he learned pest and disease identification which led him to select planting materials from the nursery farm. Previously, he was just getting the planting materials without any selection. In 2016/17, he further multiplied the materials he obtained from the demonstration plot and managed to sell 450 bundles at MK800 (US$1.10) per bundle – something that had never happened in the past. Being a lead farmer, this knowledge is being shared with other farmers.

Networking

Through the project, DARS has strengthened institutional networks with other project implementing partners within Africa as well as well-known institutions abroad. Among the latter are Rutgers University (USA), DSMZ Plant Virus Department (Germany), Makerere University (Uganda), NCSU, Department of Agriculture and Food Western Australia and UWA and Virology Laboratory, CIAT (Colombia). Such collaborations have raised the profile of the work carried out in Malawi and has

been a source of encouragement for young scientists to work in their own country and know that they can access up-to-date knowledge in their field.

Learning outcomes

Cassava production techniques

The CDP has strengthened farmers' knowledge on cassava production techniques especially on CMD and CBSD identification, management and control. This has significantly increased farmers' cassava yields, sustained their livelihoods and improved their economic strength. This also has promoted several businesses and opportunities for smallholder famers (details under Impacts above).

Diagnostics and control of viral pathogens

The characterization of viruses and disease vectors has improved researchers' knowledge on biology of viruses associated with cassava in the country and beyond. This is ideal for designing detection and diagnostics methods for control of viral pathogens.

Surveillance and monitoring of disease spread

Through the project, it has been established that cassava viruses continue to migrate from one place to another in East and Central Africa. This knowledge has helped scientists and policy makers to strengthen surveillance and monitoring of disease spread. It has also helped in advancing phytosanitary measures when distributing planting materials from one region to another. Furthermore, disease and virus distribution maps, developed during the project, have assisted scientists to make decisions on where to multiply clean seed and where to plant demonstration plots.

Laboratory documentation

The CDP improved our skill and expertise in documenting laboratory experiments. The laboratory technicians now have a well-organized way of documenting their work, unlike previously. This has also been applied to other non-CDP projects/activities currently being implemented in the laboratory.

Conclusion

The project has added to the country's knowledge about cassava viral diseases. We have provided guidelines for breeders, plant pathologists and other stakeholders in the cassava value chain and seed systems with guidelines on selection and deployment of clean cassava planting materials for food security and income generation among smallholder farmers in Malawi. With respect to CBSVs, our studies have contributed to the molecular and epidemiological knowledge of the viruses that cause the disease by establishing their rate of evolution and understanding their migration and phylogeography in East and Central Africa.

Our molecular findings have also highlighted the existence of a distinct subspecies/strain of CBSV, which was tentatively called CBSV-Tanzania. By depositing our sequences in the public domain (GenBank), we have contributed to further studies on CBSV biology and genetics. The findings of our

project have resulted in an increased body of knowledge that can provide an important input into disease control and monitoring programs.

A valuable extension to the work carried out in this project could be the use of deep sequencing techniques to unravel the novel viruses that might exist in cultivated as well as non-cultivated plants and establish evolutionary relationships between those viruses and cassava viruses identified to date.

Publication of research findings

Manuscripts submitted for publication 2018

Mbewe, W., Hanley-Bowdoin, L., Ndunguru, J. and Duffy, S. (2018) Cassava viruses – host jumps, virus recombination, spread in plant material. *Emerging Plant Diseases and Global Food Security* (in press).

Theses submitted 2018

Mtonga, A. MSc Thesis submitted to Makerere University. *Molecular characterization of Geminiviruses infecting cassava in Malawi*.

Manuscripts under internal review 2018

Mbewe, W., Mukasa, S.B., Tairo, F., Sseruwagi, P., Ndunguru, J. and Duffy, S. Evolutionary history of cassava brown streak virus in East Africa.

Mbewe, W., Pankomera, P., Mhone, A., Mwase, R., Benjala, M. Mtonga, A. Chilungo, S. Benesi, I. Sseruwagi, P., Tairo, F. and Ndunguru, J. Occurrence and spread of cassava brown streak and associated viruses in Malawi 2013 to 2015

Mtonga, A.P., Mbewe, W.K., Mukasa, S.B., Benesi, I.R.M., Mhone, A., Sseruwagi, P., Ndunguru, J. and Tairo, F. A review of cassava mosaic disease status and its associated virus species in Malawi.

Mtonga, A.P., Mbewe W.K., Mukasa, S.B., Benesi, I.R.M., Mhone, A., Sseruwagi, P., Ndunguru, J. and Tairo, F. Small RNA deep sequencing-based detection of cassava viruses: First report of Cassava Virus C in Malawi.

Mtonga, A.P., Mbewe, W.K., Mukasa, S.B., Benesi, I.R.M., Mhone, A., Sseruwagi, P., Ndunguru, J. and Tairo, F. Genetic variation in DNA-B component of cassava mosaic begomoviruses in Malawi.

Manuscripts published 2017

Mbewe, W., Tairo, F., Sseruwagi, P., Ndunguru, J., Duffy, S., Mukasa, S.B., Benesi, I., Sheat, S., Koebler, M. and Winter, S. (2017a). Variability in P1 gene redefines phylogenetic relationship among Cassava brown streak viruses. *Virology Journal*, 14(1):118.

Mbewe, W., Winter, S., Mukasa, S.B., Tairo, F., Sseruwagi, P., Ndunguru, J. and Duffy, S. (2017b). Deep sequencing reveals a divergent strain of Ugandan cassava brown streak virus isolated from Malawi. *Genome Announcements*, 5(33):e00818-17.

Conference presentations

Mbewe, W., Mukasa, S.B., Tairo, F., Sseruwagi, P., Ndunguru, J., Duffy, S. *High rates of evolution and pervasive purifying selection in coat protein gene of cassava brown streak virus.* Rutgers University Microbiology Symposium, 6 – 9th February 2017, New Brunswick, New Jersey, USA.

Mbewe, W., Duffy, S. *Comparative evolvability of plant viruses*. American Society of Microbiology Microbe Symposium, 1 – 5 June 2017. New Orleans, Louisiana, USA.

Mbewe, W., Mukasa, S.B., Tairo, F., Sseruwagi, P., Ndunguru, J., Duffy, S. *Rates of evolution and pervasive purifying selection among Cassava brown streak viruses*. International Plant and Animal genome Conference (PAG XXV), 14 – 18th January 2017, San Diego, California, USA.

Mbewe, W., Mukasa, S.B., Tairo, F., Sseruwagi, P., Ndunguru, J., Duffy, S. *Temporal structure and rates of evolution in the coat protein gene of cassava brown streak virus*. 9th Virus Evolution Workshop, 9 – 11th March 2017, Pennsylvania State University, PA, USA.

Mbewe, W., Tairo, F., Sseruwagi, P., Ndunguru, J., Duffy, S., Mukasa, S.B., Benesi, I., Sheat, S., Koebler, M., Winter, S. *Variability in P1 gene redefines phylogenetic relationship among Cassava brown streak viruses*. World congress on Root and Tuber Crops. January 2016. Nanning, China.

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