KENYA

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Abstract

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

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

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

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

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

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

Acronyms and abbreviations

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

Results summary: Kenya

Aim I: Understand the threat from evolving viruses and vectors

Objective 1: Disease epidemiology

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

Objective 2: Characterization of emerging viruses

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

Objective 3: Characterization of disease vectors

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

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

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

Background

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

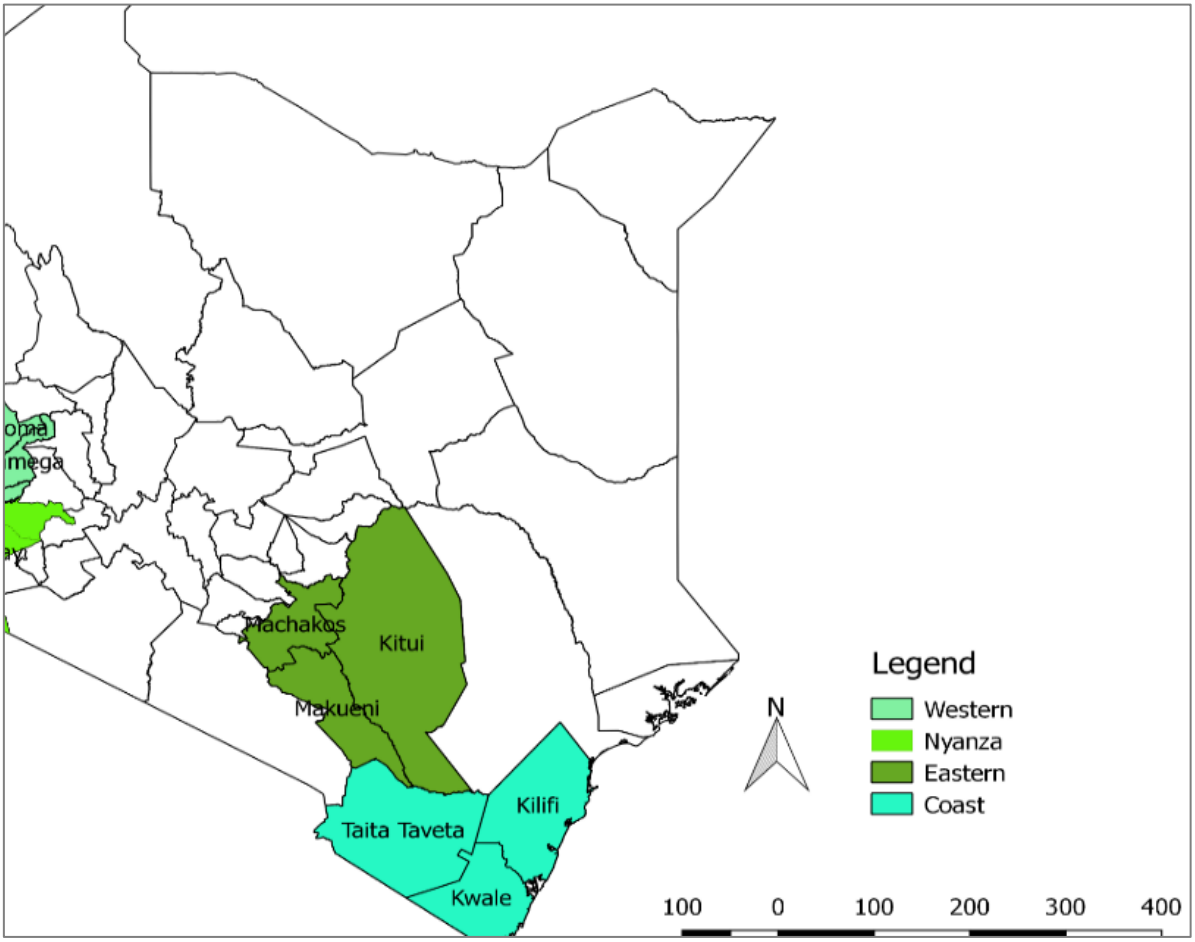


Figure 1 Cassava cultivation areas in Kenya

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

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

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

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

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

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

SECTION ONE: Understanding the threat from evolving viruses and vectors

Disease epidemiology

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

CMD and CBSD incidence

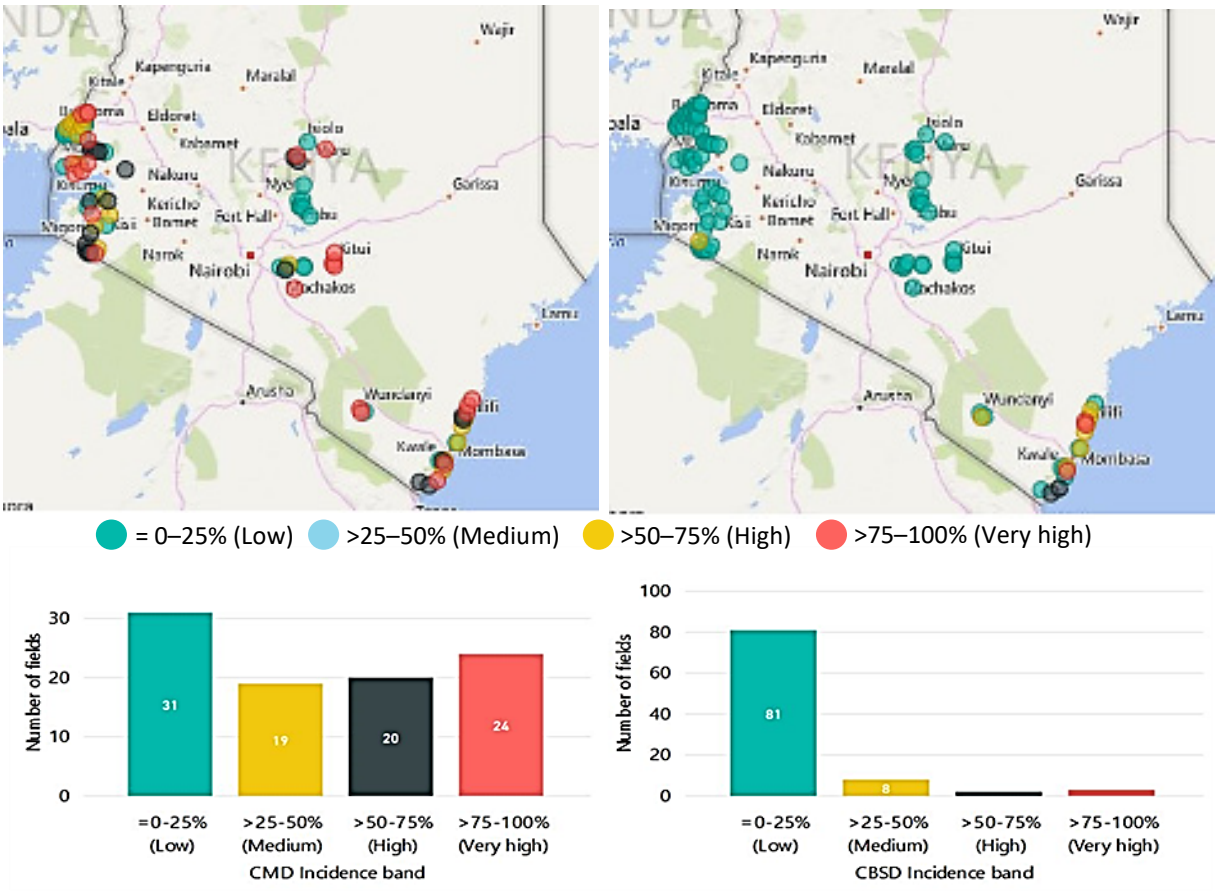


Figure 2 CMD (left) and CBSD (right) 2009 survey: locations sampled and calculated mean incidence at the locations

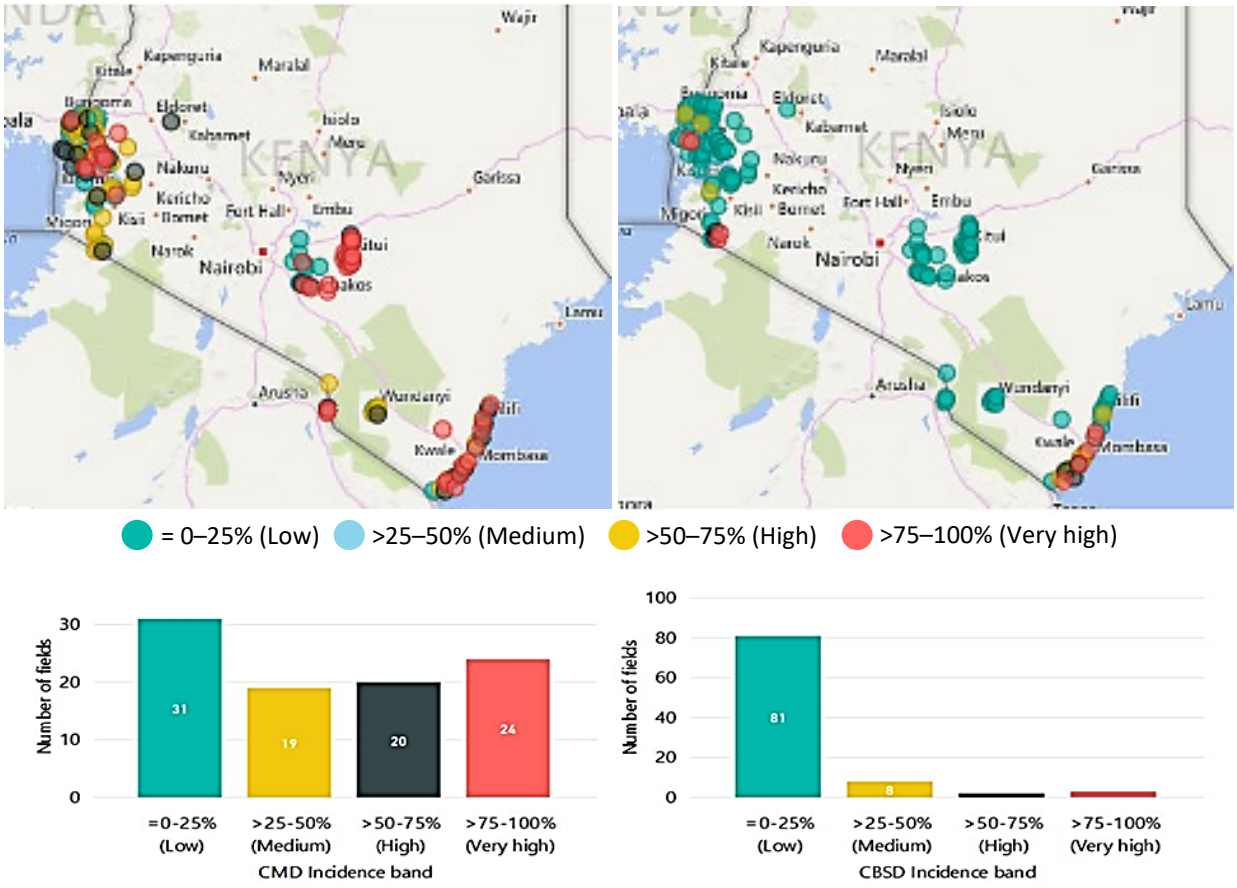


Figure 3 CMD (left) and CBSD (right) 2015 survey: locations sampled and calculated mean incidence at the locations

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

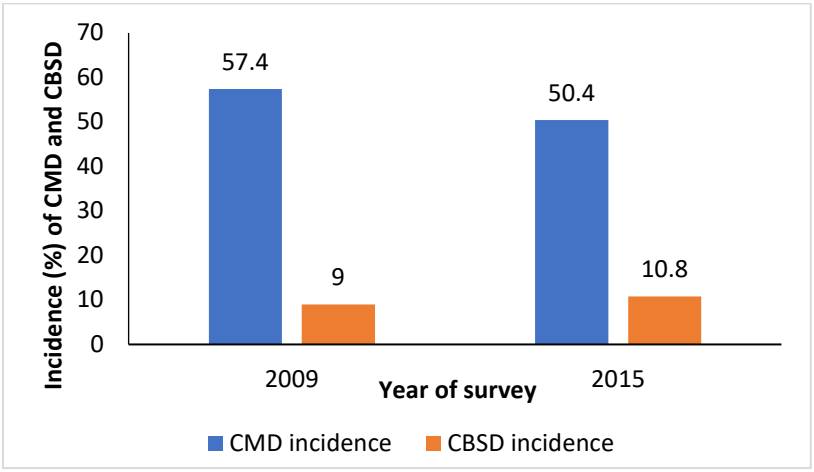


Figure 4 Mean CMD and CBSD incidence, 2009 and 2015

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

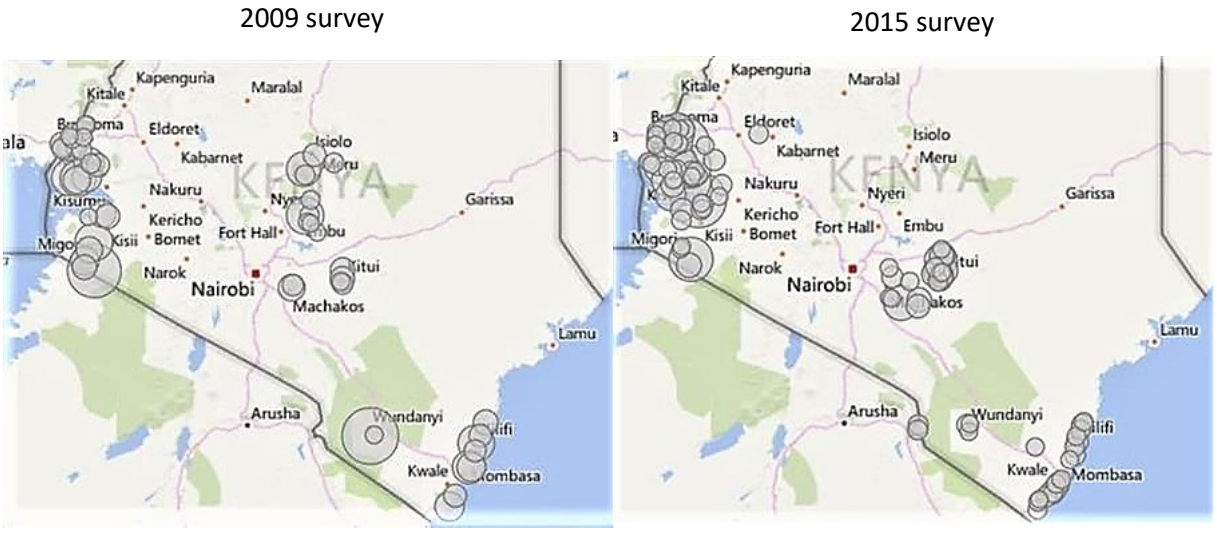


Figure 5 Distribution of whitefly population – based on locations surveyed. Grey circle sizes reflect the number of farms sampled

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

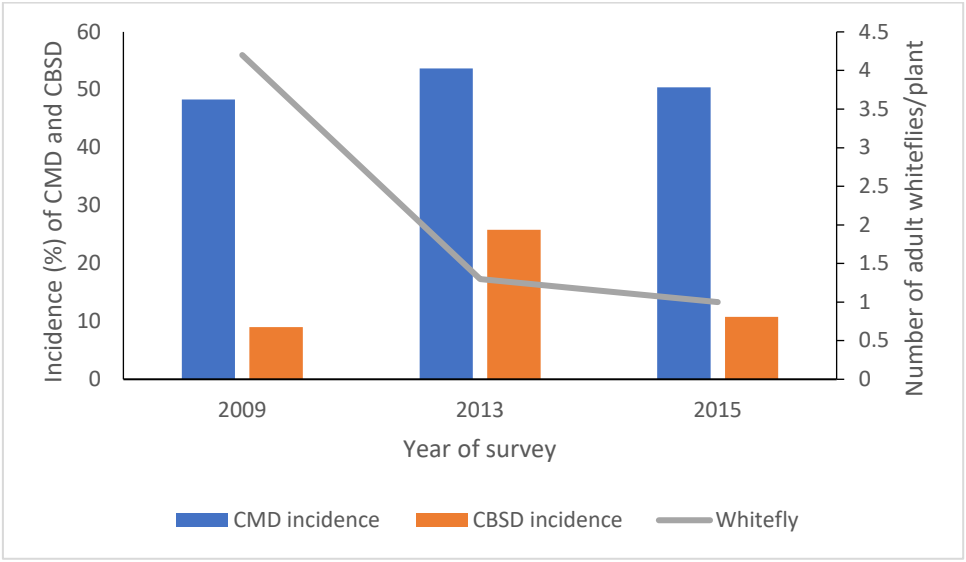
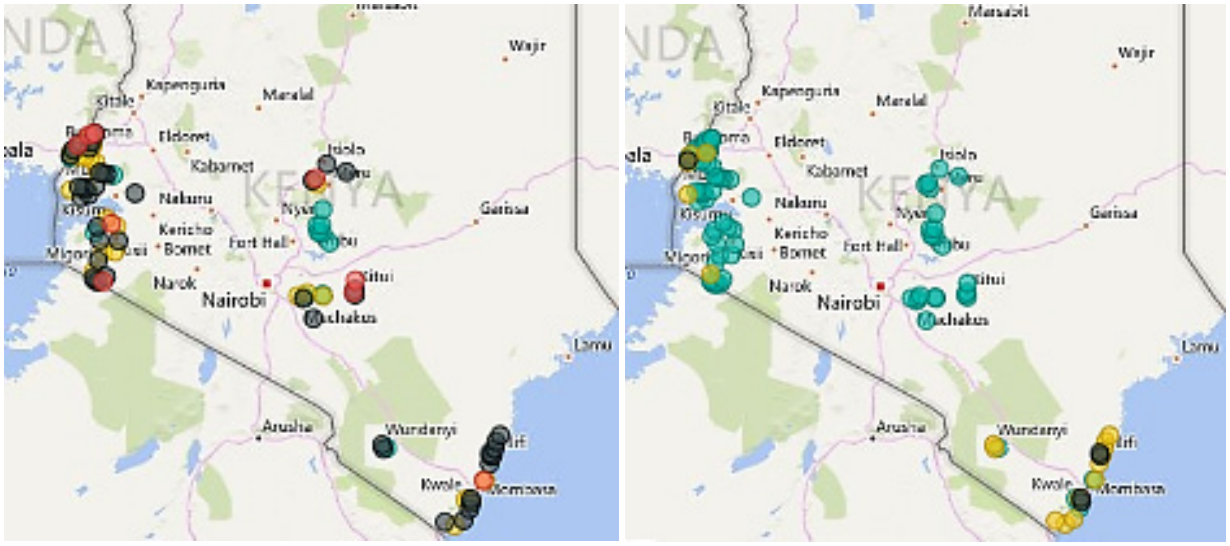


Figure 6 CMD, CBSD and whitefly abundance trends 2009–2015

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

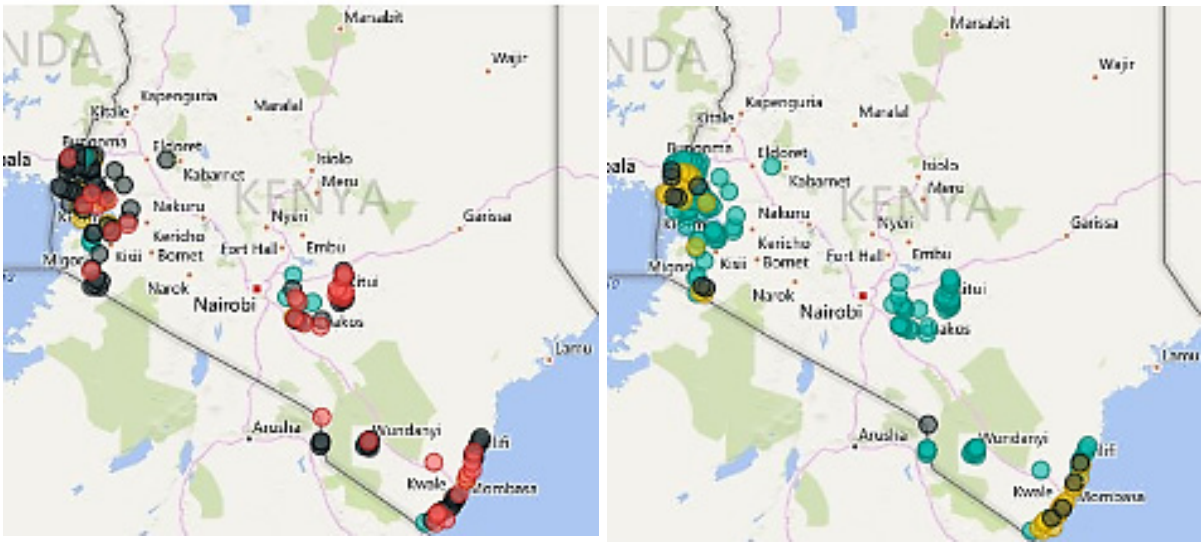
The recorded CMD and CBSD severity for the regions surveyed is shown in Figure 7 and Figure 8 for the same period.

CMD and CBSD severity



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

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



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

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

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

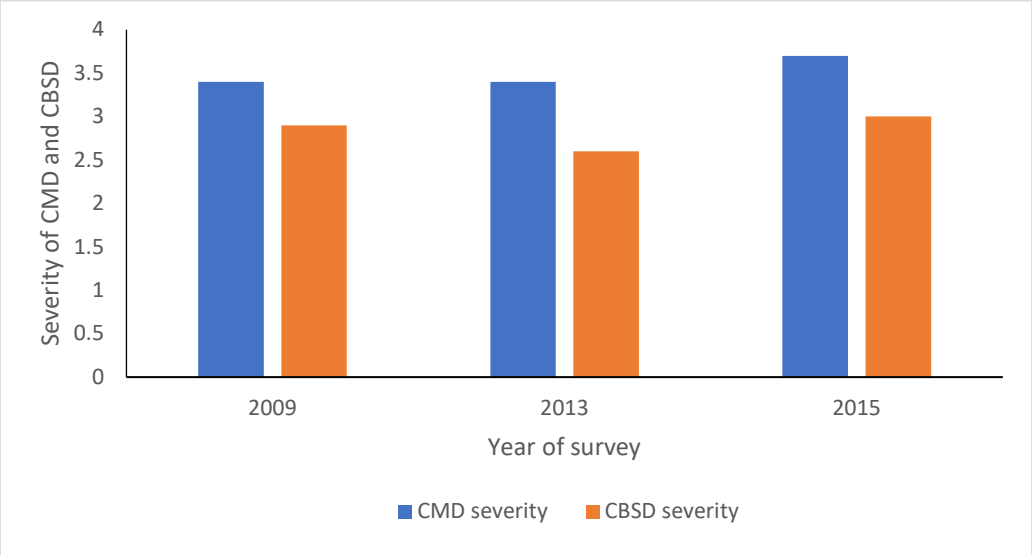


Figure 9 Mean CMD and CBSD severity trend 2009–2015

Detection of cassava-infecting viruses

CMBs

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

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

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

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

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

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

CBSVs

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

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

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

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

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

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

Genetic diversity of CMBs in Kenya

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

Genetic diversity of CBSVs

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

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

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

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

Interaction between CBSVs and CMBs and its impact on disease development

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

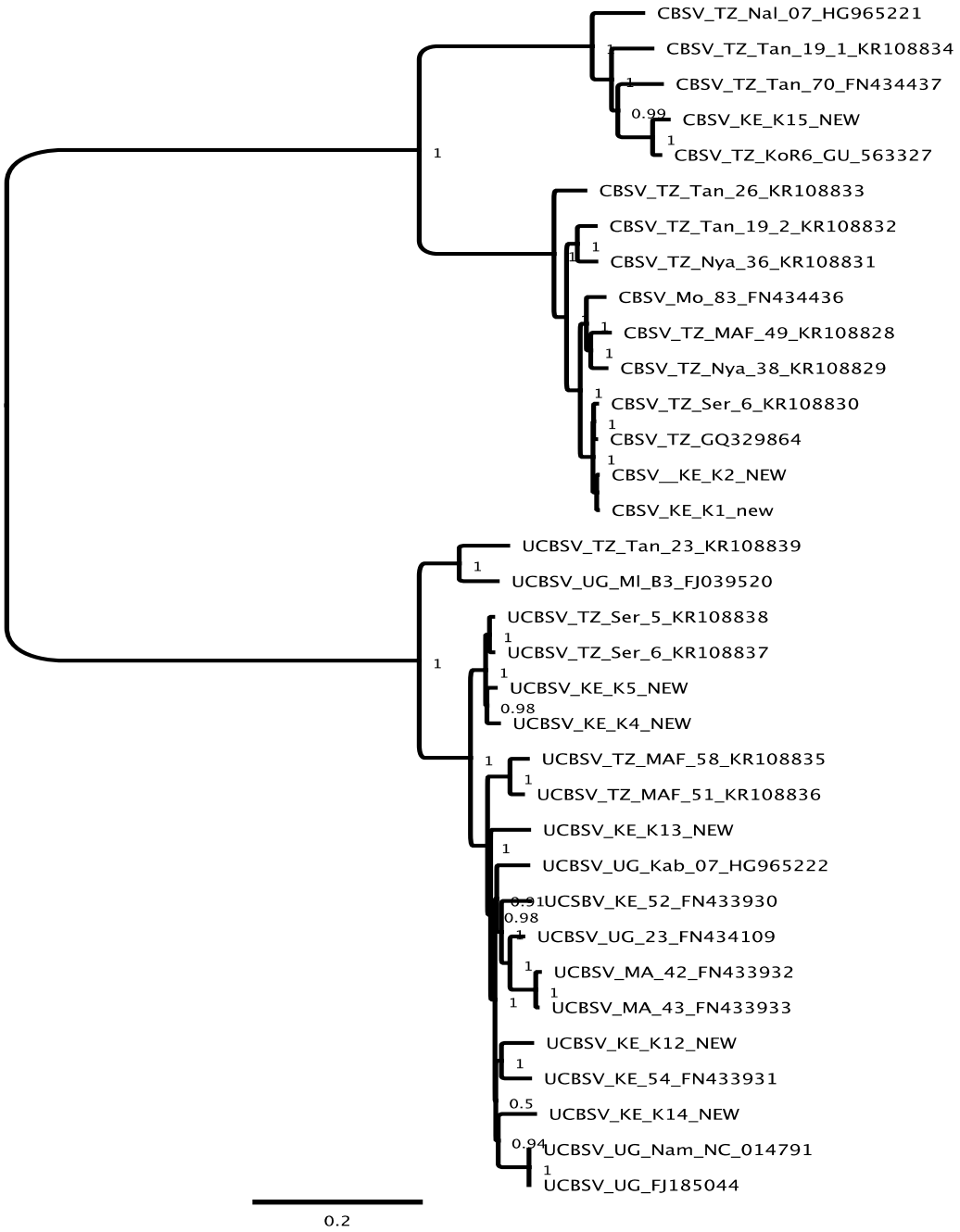


Figure 10 Phylogenetic tree of whole CBSV/UCBSV genomes (nucleotides) following Bayesian analyses using ExaBayes (adapted from Ateka et al., 2017)

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

Determination of alternative hosts for CMBs and CBSVs

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

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

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

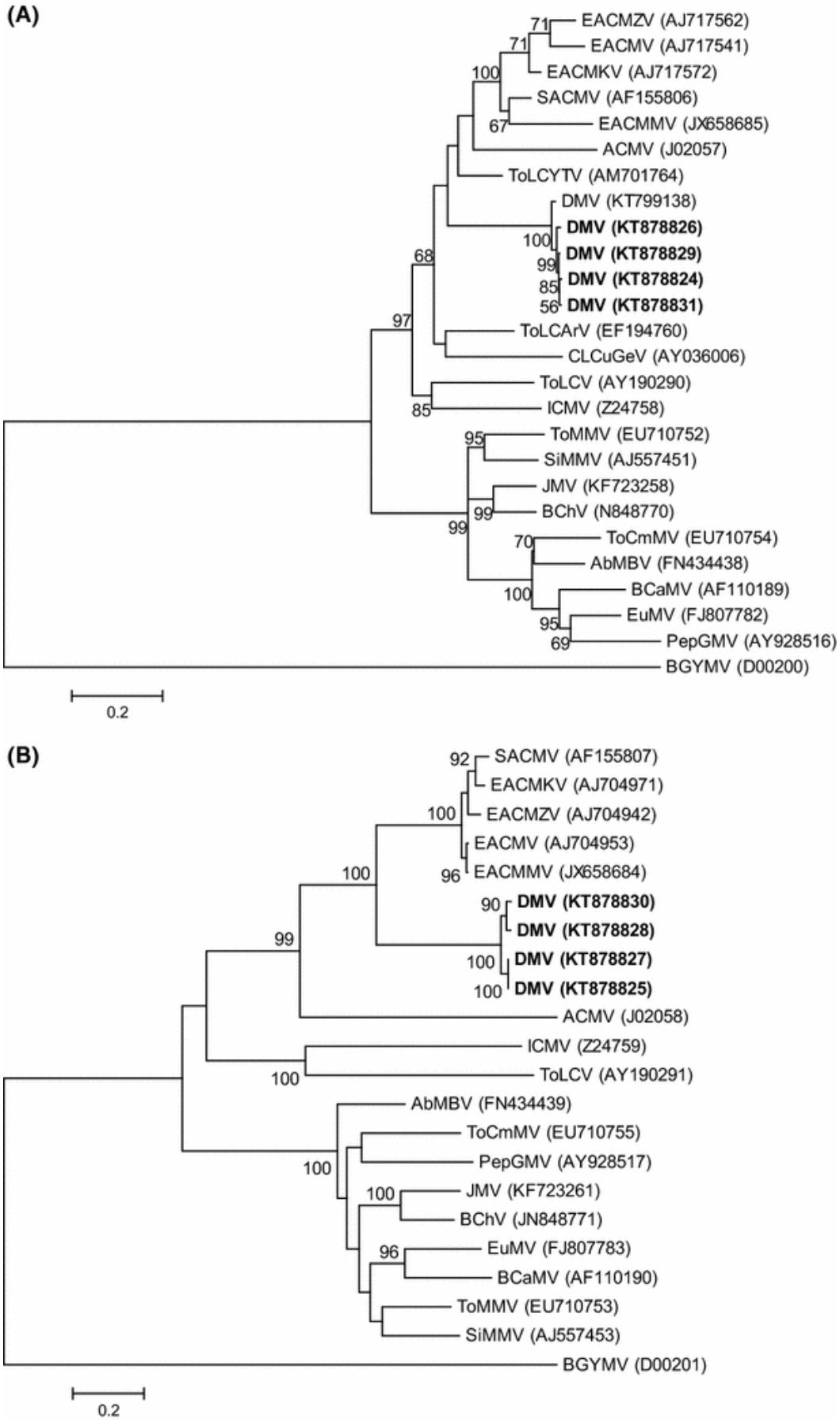


Figure 11 Maximum-likelihood trees inferred from the alignment of full-length DNA-A (A) and DNA-B (B) nucleotide sequences of DMV (in bold) and selected begomoviruses (adapted from Kyallo et al., 2017)

Characterization of disease vectors

Genetic diversity of *Bemisia tabaci* on cassava

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

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

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

(Source: Manani et al., 2017)

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

Development of diagnostic tools

Development of a loop-mediated isothermal amplification assay CBSVs

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

SECTION TWO: Integrated pest management in Kenya

Conventional breeding support

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

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

Figure 12 Project team visiting breeders' material at KALRO Mtwapa

Incidence and severity of CMD and whitefly populations

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

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

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

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

Incidence and severity of CBSD

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

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

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

Reaching farmers directly and through partners

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

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

Field training for disease surveillance surveys

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

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

Training at agricultural shows and exhibitions

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

Table 9 Number and categories of visitors at public events

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Figure 19 Farmers receiving cuttings from demonstration farms

Information materials developed and disseminated

Communications – creating awareness

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

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

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

Reaching farmers indirectly through partners

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

Table 13 Number of extension officers trained in different counties

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

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

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

Outputs from the training

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

Build sustainable regional capacity

Strengthening stakeholder linkages

Stakeholder engagement

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

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

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

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

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

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

Strengthening human capacity and infrastructure

Human capacity

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

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

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

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

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

Table 16 Postgraduate students supported by CDP – Kenya

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

Staff training in advanced labs

Training in disease epidemiology and modeling

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

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

Bioinformatics training

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

Recruitment of project staff

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

Table 17 Personnel recruited by CDP Kenya and respective roles

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

Infrastructure strengthening

Laboratory and greenhouse construction

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

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

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

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Hope for Cassava Farmers as JKUAT Unveils Diagnostics Lab

Posted on October 5, 2016 by Corporate Communications Office

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

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

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

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

News & Events

AFRICA-ai-JAPAN Project Gets an Extension

Network to Enhance Administration of Third Party Funding in Universities

Varsity Automates Students' Hostel Application

Information for Prospective Students

Admitted KUCCPS Students – SEPTEMBER INTAKE 2018

Online Application and Admission Letters

Online Admissions Video Tutorial

Admission into Distant/Online

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

Equipment procured by the project in JKUAT Lab

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

Table 18 Assets purchased by CDP in Kenya and their description

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

SECTION THREE: Impacts, success stories and learning outcomes

Impacts

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

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

Success stories

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

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

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

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

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

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

Learning outcomes

Change in farming practices

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

Increased effectiveness of extension officers

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

Plant breeding and disease management

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

Strengthened linkages

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

Data collection

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

Laboratory management

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

Conclusion

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

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

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

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

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

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

Publication of research findings

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Manuscripts in preparation

Mwaura et al., Prevalence of cassava brown streak disease (CBSD) and associated viruses in Kenya over eight years: 2009–2017. In preparation.

Muga et al., Diversity and distribution of cassava mosaic begomoviruses in Kenya. In preparation.

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

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