RWANDA

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Abstract

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

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

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

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

Acronyms and abbreviations

ACMV	African cassava mosaic virus
CBSD	Cassava brown streak disease
CBSV	Cassava brown streak virus
CDP	Cassava Diagnostics Project
СМВ	Cassava mosaic begomoviruses
CMD	Cassava mosaic disease
CTL	Country Team Leader
EACMV	East African cassava mosaic virus
EACMV ISAR	<i>East African cassava mosaic virus</i> Institut des Sciences Agronomiques, du Rwanda
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ISAR	Institut des Sciences Agronomiques, du Rwanda
ISAR MAP	Institut des Sciences Agronomiques, du Rwanda Months after planting
ISAR MAP RAB	Institut des Sciences Agronomiques, du Rwanda Months after planting Rwanda Agricultural Board

Results summary: Rwanda

Aim I: Understand the	threat from evolving viruses and vectors
Objective 1: Disease epide	miology
Disease and whitefly prevalence surveys conducted	 Four surveys conducted in 2009, 2013, 2015 and 2017, covering 410 fields in 14 districts. Survey data for 2009, 2013 and 2015 were cleaned and validated by Tanzania Agriculture Research Institute (TARI)– Mikocheni and Cambridge modeling teams during the data entry and assembly workshop held from 27 November to 3 December 2016 in Dar es Salaam, Tanzania. A new SOP for 2017 countywide survey developed and used to collect 2017 data. Cassava leaf samples with Cassava mosaic disease (CMD) and Cassava brown streak disease (CBSD)-like symptoms, were collected for laboratory analysis.
Relationship between CBSVs presence/load and disease symptom development determined	 One manuscript published by Esperance Munganyinka from her PhD research (Munganyinka et al., 2018). Participated in the AgShare.Today Scientific Writing Workshop in Lusaka, 2–7 October 2017. Currently engaged on Objective 2 to quantify the viral loads of <i>Cassava brown streak virus</i> (CBSV)-infected plants in the DSMZ laboratory under the supervision of Dr Stephan Winter in Germany.
Spread of CBSD within and between cassava fields	 Ms Jeanine Umfuyisoni defended her MSc dissertation successfully in 2018 at Makerere University.
Alternative hosts for CBSVs and CMBs and associated insect vectors identified	 CBSV whole genome sequences obtained from non-cassava shrubs in Rwanda as part of Ms Jeanine Umfuyisoni's MSc research. One manuscript by Ms Umfuyisoni is in preparation to report on her MSc research.
Objective 2: Characterizati	ion of emerging viruses
Cassava virus isolates in the project countries sequenced and analyzed	• CBSV and Uganda cassava brown streak virus (UCBSV) whole genomes were obtained and their genetic diversity studied.
Cassava virus distribution maps for partner countries, generated (incidence, severity, whitefly)	Disease distribution maps produced and distributed to stakeholders.
Objective 3: Characterizati	
TASKS Whiteflies characterized	 RESULTS Whitefly samples were collected and characterized – identifying the Rwanda species as SSA1.

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

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

Background

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

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

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

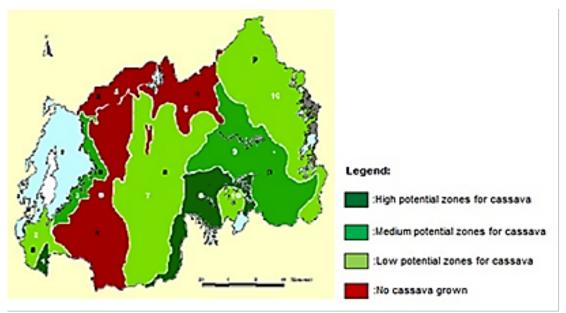


Figure 1 Major agro-ecological zones in Rwanda (adapted from Bazarusanga et al., 2011)

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

SECTION ONE: Understanding the threat from evolving viruses and vectors

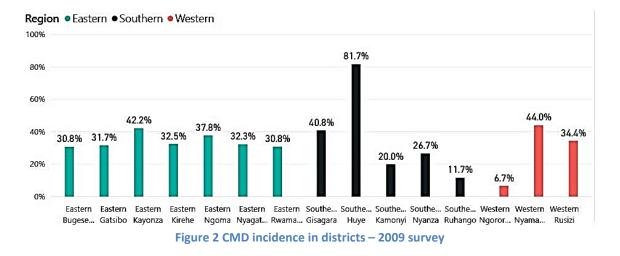
Disease epidemiology

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

CMD incidence and symptom severity

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

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



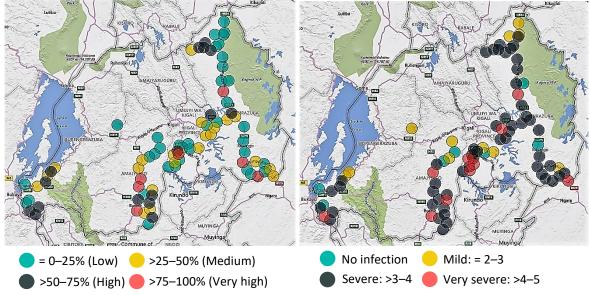
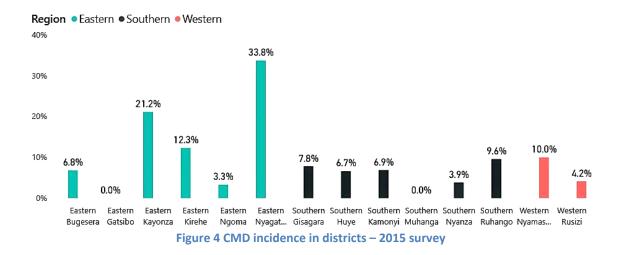
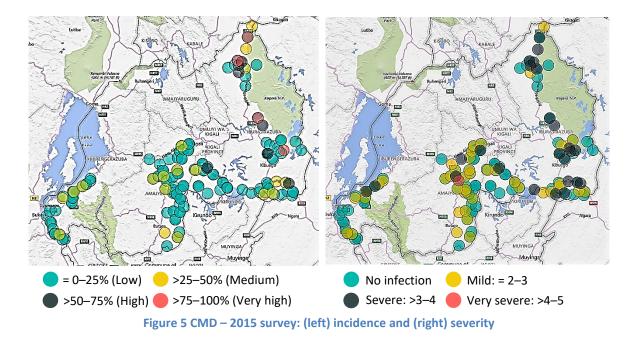


Figure 3 CMD – 2009 survey: (left) incidence and (right) severity

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





CBSD incidence and severity

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

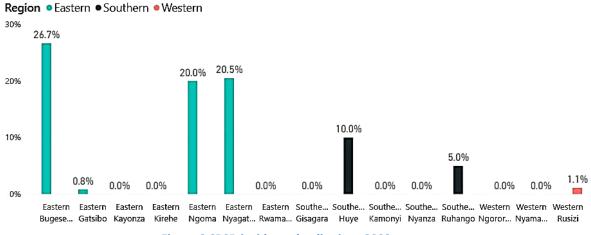


Figure 6 CBSD incidence by district – 2009 survey

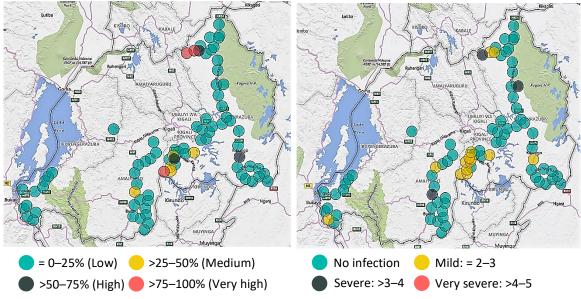
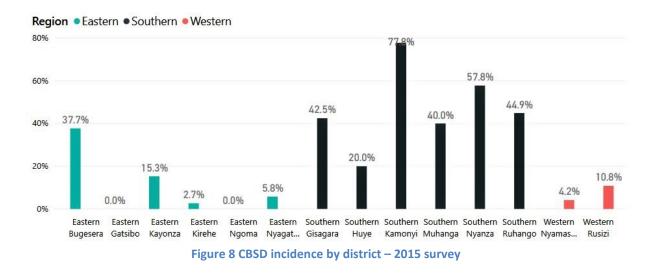
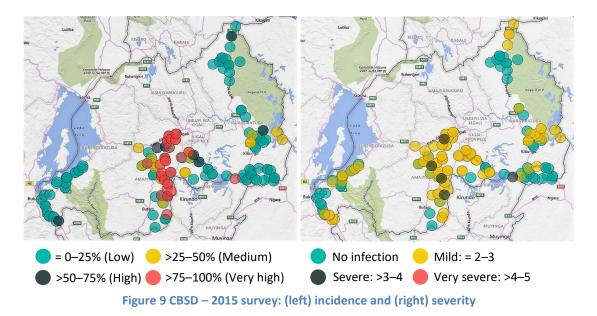


Figure 7 CBSD – 2009 survey: (left) incidence and (right) severity

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

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





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

Characterization of emerging viruses

Cassava virus isolates sequenced and analyzed

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

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

Table 1 Detection of CBSVs in Rwanda, 2015

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

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

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

Epidemiology and distribution of CBSD in Rwanda

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

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

The relationship between CBSV presence and disease development

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

Characterization of disease vectors

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

SECTION TWO: Integrated pest management

Conventional breeding support

Breeders' material monitored for disease

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

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

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

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

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

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

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

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

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

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

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

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

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

Reaching farmers directly and through partners

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

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



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



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

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

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

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

Table 8 Demonstration plot harvest in Bugesera (80 m²)

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

Table 9 Demonstration plot harvest in in Ruhango (80m²)

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

Table 10 Dissemination of cuttings in Rweru Sect	tor of Bugesera District in Rwanda
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Sector	Sites	Villages	No. of male	No. of female	No. of cuttings received
Rweru	Nemba	10	6	4	1000
	Batima	4	0	4	400
	Total	14	6	8	1400

Information materials developed and disseminated

Training

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

Awareness

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

Participation in agriculture show

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



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

Build sustainable regional capacity

Project inception and consultative meeting with stakeholders

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

Stakeholder engagement

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

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

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

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

Exchange visits between scientists in the project countries

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

Outreach to regional virologists in non-project countries

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

Strengthening human capacity and infrastructure

Human capacity staff in Rwanda

Project staff recruited

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

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

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

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

The objectives of the visit were:

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

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

Infrastructure strengthening

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

SECTION THREE: Impacts, success stories and learning outcomes

Impacts

This information was not available at the time of writing.

Success stories

This information was not available at the time of writing.

Learning outcomes

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

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

List of manuscripts

- Munganyinka, E., Ateka, E.M., Kihurani, A.W., Kanyange, M.C., Tairo, F., Sseruwagi, P. and Ndunguru, J. (2017) Cassava brown streak disease in Rwanda, the associated viruses and disease phenotypes. *Plant Pathology*, 67:377–387.
- Munganyinka, E., Margaria, P., Sheat, S., Ateka, E.M., Tairo, F., Ndunguru, J. and Winter S. (2018) Localization of cassava brown streak virus in *Nicotiana rustica* and cassava *Manihot esculenta* (Crantz) using RNAscope[®] *in situ* hybridization. *Virology Journal*, 15:128.
- Nyirahorana, C., Mburu, D. M., Mulyungi, P., Ntaganira, E., Ndunguru, J., Sseruwagi, P., Kanyange,
 M.C. and Nsengiyumva, A. (2017) Drivers behind adoption of Cassava brown streak disease
 control measures in Rwanda. *International Journal of Scientific & Technology Research*,
 6(11):113–117.

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References

- Bazarusanga, T., Marcotty, T., Ahouandjinou, A.M.K.I., Ntumba, T., Katendi, C. and Geysen, D. (2011)
 Estimation of the *Theileria parva* entomological inoculation rate (EIR) by means of tick
 burden and proportion of infected questing ticks in three different farming systems in
 Rwanda. *International Journal of Vocational and Technical Education*, 3(7):99–106.
- Mbanzibwa, D.R., Tian, Y.P., Tugume, A.K., Mukasa, S.B., Tairo, F., Kyamanywa, S., Kullaya, A., Valkonen J.P. (2009) Genetically distinct strains of Cassava brown streak virus in the Lake Victoria basin and the Indian Ocean coastal area of East Africa. *Archives of Virology*, 154(2):353-9. doi: 10.1007/s00705-008-0301-9. Epub 2009 Jan 30.