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Full Length Research Paper

Changes in the epidemiology of cassava brown streak disease and associated viruses in Rwanda: occurrence and distribution since 2009

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Cassava brown streak disease (CBSD), the most important viral disease of cassava in Africa is caused by two ipomoviruses, *Cassava brown streak virus* (CBSV) and *Ugandan cassava brown streak virus* (UCBSV). Field surveys were conducted in 2009, 2013, 2015 and 2017 to determine the incidence and severity of CBSD in major cassava growing districts of Rwanda. Farmers' fields were assessed and leaf samples collected for virus detection using RT-PCR. Results showed that the disease occurred in Southern province with an average incidence of 60.8% and severity of 2.5, Eastern province with 39.2% and 2.5 and Western Province with 22.2% and 3.2. The highest incidence was recorded in Southern Province (60.8%) and the lowest in Western Province (22.2%). The mean severity ranged from 2.0 to 2.8 on the surveyed cassava plants. RT-PCR detected both CBSV and UCBSV species, which occurred in single CBSV (10%), UCBSV (9.6%) and dual CBSV + UCBSV (1.6%) infections. The study showed the widespread occurrence of CBSD, its severity and the associated viruses in Rwanda. It highlights the urgency of implementing effective control measures to avert the impact of the disease on the food crop that feeds most households in the country.

Keywords: Cassava, CBSD, CBSVs, incidence, severity and epidemiology.

Abbreviations

CBSD: Cassava brown streak disease
CBSV: Cassava brown streak virus
CMD: Cassava mosaic disease
CTAB: Cetyltrimethylammonium bromide
DNA: Deoxyribonucleic acid
DRC: Democratic Republic of Congo
MARI: Mikocheni Agricultural Research Institute
PCR: Polymerase chain reaction
RAB: Rwanda Agriculture and Animal Resources Development Board
RNA: Ribonucleic acid
RT-PCR: Reverse transcriptase polymerase chain reaction
UCBSV: Ugandan cassava brown streak virus
UV: Ultraviolet

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a staple food for approximately 500–800 million people living in developing countries and worldwide (Bull *et al*., 2011; Howeler *et al*., 2013). It is second only to maize (*Zea mays* L) for production of starch (Howeler *et al*., 2013).

In 2018, the worldwide production was estimated at 277 MT

while it was 152 MT in Africa (FAO, 2018). In the developing world, cassava is among the top four most important crops in terms of production after rice, maize and wheat. The potential yield of cassava is estimated at 90 t/ha of fresh roots under well-managed conditions (El-Sharkawy, 2004). Cassava plays a key role as a food security (FAO, 2018; Waisundara, 2018).) and income-generating food crop for many smallholder farmers in developing countries (Ceballos et al., 2004; El-Sharkawy, 2004; Cassava is also recognized as a resilient crop that can strive in drought conditions particularly in eastern and southern African countries that regularly suffer continuous periods of drought (FAO, 2018, Waisundara, 2018).

In East Africa, cassava is eaten after boiling and processing to flour to make porridge, local brew, ugali and bread. Sweet varieties lacking cyanogenic glycosides can be eaten raw (Kamau, 2006; Mkumbira *et al*., 2003; Were, 2011). In addition, cassava can be used in industries for production of animal feed and starch for use in pharmaceuticals, textiles and more (Ceballos *et al*., 2004; El-Sharkawy, 2004).

In Rwanda, cassava is an important staple food and is currently being promoted as a cash crop feeding cassava processing plants. In addition to its tuberous roots, its leaves are consumed as a popular vegetable called 'Isombe'. 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 (mixed with beans, boiled, paste or ugali etc.). It occupies the third place after banana and sweet potato for reducing hunger and poverty in the country (FAOSTAT 2011; Night *et al*., 2011) and its production was 1041843 tonnes in 2018 (FAOSTAT, 2018). 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).

Cassava brown streak disease constitutes the most severe threat to the production of cassava in East and Central Africa. CBSD is caused by two ipomoviruses: *Cassava brown streak virus* (CBSV) and *Ugandan cassava brown streak virus* (UCBSV) (Mbanzibwa *et al.,* 2011). CBSD has spread beyond the limits of its previously confined distribution in coastal East Africa (Nichols, 1950), to affect large parts of the Great Lakes Region of East and Central Africa (Alicai *et al*., 2007; Mbanzibwa *et al*., 2011). CBSD was first described at the Amani Research Centre in north-eastern Tanzania. The disease remained endemic in lowland regions of coastal East Africa and lakeshore areas of Malawi, until 2004 when new outbreaks were reported from altitudes above 1000 m.a.s.l in Uganda (Alicai *et al.,* 2007). As CBSD continued to spread in subsequent years, the casual viruses: CBSV and UCBSV were reported to be widespread in Uganda, Tanzania and Kenya (Mbanzibwa *et al*., 2011). The key factor driving the CMD and CBSD pandemics seems most likely to be changes in abundance of the shared whitefly vector, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) (Legg *et al.*, 2011).

In Rwanda, previous publications highlighted the threat that CBSD posed to sustainable cassava productivity, and its impact on food security and income of smallholders' farmers (Munganyinka *et al*., 2017). The study reported UCBSV to be more widespread than CBSV and it was the most important virus infecting cassava crops in Rwanda. CSBV occurred only in the Southern districts (Munganyinka *et al*., 2017). The study whose results are presented in this paper aimed to: (i) to track changes in the incidence and severity of CBSD across the country and (ii) to identify the viral species causing the disease and their geographical distribution in 2009, 2013, 2015 and 2017.

MATERIALS AND METHODS

Field assessment of incidence and severity of CBSD

Four country-wide disease monitoring surveys were carried out in 2009, 2013, 2015 and 2017 in Rwanda under phase I and II of the regional cassava virus disease diagnostics research projects, coordinated by Mikocheni Agricultural Research Institute (MARI), Tanzania. In each survey, cassava fields were selected along major roads at intervals of about 8-10 km. In each field 30 plants were scored for CBSD infection and symptom severity along two diagonals as described by Sseruwagi *et al*. (2017). Leaf samples were collected from an average of three plants per field for CBSVs analyses in molecular labs.

Leaf samples from old fields were pressed in herbarium press until analysis at Rwanda Agriculture and Animal Resources Development Board (RAB). In each field, 10 mature cassava plants were assessed for the presence or absence of CBSD root symptoms. CBSD incidence was assessed as the number of plants with disease symptoms expressed as a percentage of the total plants assessed. CBSD foliar symptoms were classified using a severity scale from 1 to 5, where 1: no visible symptoms; 2: mild vein yellowing or chlorotic blotches on some leaves; 3: pronounced/extensive vein yellowing or chlorotic blotches on leaves, but no lesions or streaks on stems; 4: pronounced/extensive vein yellowing or chlorotic blotches on leaves and mild lesions or streaks on stems; and 5: pronounced/extensive vein yellowing or chlorotic blotches on leaves and severe lesions or streaks on stems, defoliation and die back (Alicai et al. 2016).

The different survey months, number of districts covered, fields and leaf samples since 2009 are summarized in table 1.

Extraction of total RNA

Total RNA was extracted from cassava leaf samples three to four days after sampling at Musanze and Rubona Phytopathology laboratories using modified CTAB protocol. The extracted RNA was stored at -20°C before

Table 1. Different survey months, number of districts, fields and leaf samples since 2009.

Year	Months	# Districts	# Fields	Total leaf samples
2009	May	14	91	273
2013	July to August	7	70	210
2015	May to June	10	100	300
2017	June	10	100	300

RT-PCR test. The primer pairs: CBSDDF2 5'-GCTMGAAATGCYGGRTAYACAA-3' and CBSDDR 5'-GGATATGGAGAAAGRKCTCC-3' (Mbanzibwa *et al*., 2011b) were used for PCR amplification from the cDNA template.

The first strand complementary DNA was synthesized following the manufacturer's instructions (New England Biolabs). The synthesized complementary DNA was used for PCR to detect CBSVs following the manufacturer's instructions (New England Biolabs). The 25µl reaction mixture contained 5.0µl of 5x oneTaq standard buffer including 20mM $MgCl_2$, 0.5µl of 10mM dNTPs, and 0.5µl of each forward and reverse primer, 0.125µl of 5U/µl of OneTaq DNA polymerase (New England Biolabs), 2µl of cDNA template and 16.375µl of sterile distilled water. The target amplicons were amplified in thermal-cycler at 94°C for 2 min for initial denaturation, followed by 35 cycles of 94°C for 30sec, 51^{o}C for 30 sec for annealing and 72°C for 30 sec and at 72^{o}C for 10min as final extension. The PCR product was casted on 1% agarose gel stained with Ethidium Bromide. Generuler 1 Kb plus DNA ladder (thermoscientific) was used. The gel was visualized under UV light in gel image system (BioDoc-Imaging System-UVP) (Fig. 4).

RESULTS

Symptoms exhibited by CBSD-affected plants

During the countrywide surveys in 2009, 2013, 2015 and 2017, various symptoms were recorded on CBSD-affected cassava plants in the surveyed fields. Leaf symptoms included: yellow patches, chlorotic spots, chlorotic blotches, pronounced mottling and veinal and interveinal chlorosis along the secondary and tertiary veins, which occurred mainly on the lower older leaves. Stem symptoms manifested as scratch-like wounds, dark brown spots, and streaks. Various levels of root constrictions were also recorded that combined with various discolorations (brown, black or yellow, or chalky) in tuberous roots (Fig. 1. A to H).

Incidence of CBSD

In general, mean CBSD leaf incidence ranged from 5.8% to 37.1% over the nine years (2009 to 2017) of field assessments. Based on foliar disease symptoms, CBSD incidence was 13.2% in 2009. Disease incidence declined to the lowest record mean of 5.8 % in 2013, and increased to 37.1% and 28.8% in 2015 and 2017, respectively (Fig.2). In 2009, foliar disease incidence was high in Nyagatare (24.1%) and Bugesera (23.4%) districts, Eastern province; it was low in Gisagara (5%) and Huye (5%) districts, Southern province (Fig. 2 A).

In 2013, foliar disease incidence was mild in Nyanza (10%) district, Southern province and low in Kirehe (4%) district, Eastern province. Based on CBSD root necrotic symptoms, CBSD incidence was highest in Bugesera (65%), Kirehe (62%) and Nyagatare (60%) districts, Eastern province, and Ruhango (45%) district, Southern province (Fig. 2 B).

In 2015, foliar disease incidence was very high in Kamonyi (86%), Nyanza (64%) and Ruhango (54%) districts, Southern province. It was very low in Nyagatare (4%) district, Eastern province and mild in Nyamasheke (10%) district, Western province.

Based on CBSD root necrotic symptoms, CBSD incidence was highest in Kamonyi (46%), Ruhango (48%), Nyanza (46%) and Gisagara (38%) districts, Southern province and Bugesera (36%) district, Eastern province (Fig. 2 C).

In 2017, foliar disease incidence was high in Bugesera (40%), moderate in Kirehe (20%) districts, Eastern province, high in Kamonyi (30%), Nyanza (33%) and Ruhango (25%) districts, Southern province. Based on CBSD root necrotic symptoms, CBSD incidence was highest in Bugesera (68%), Kirehe (40%) districts, Eastern province and Nyanza (53%), Kamonyi (30%) districts, Southern province (Fig.2 D).

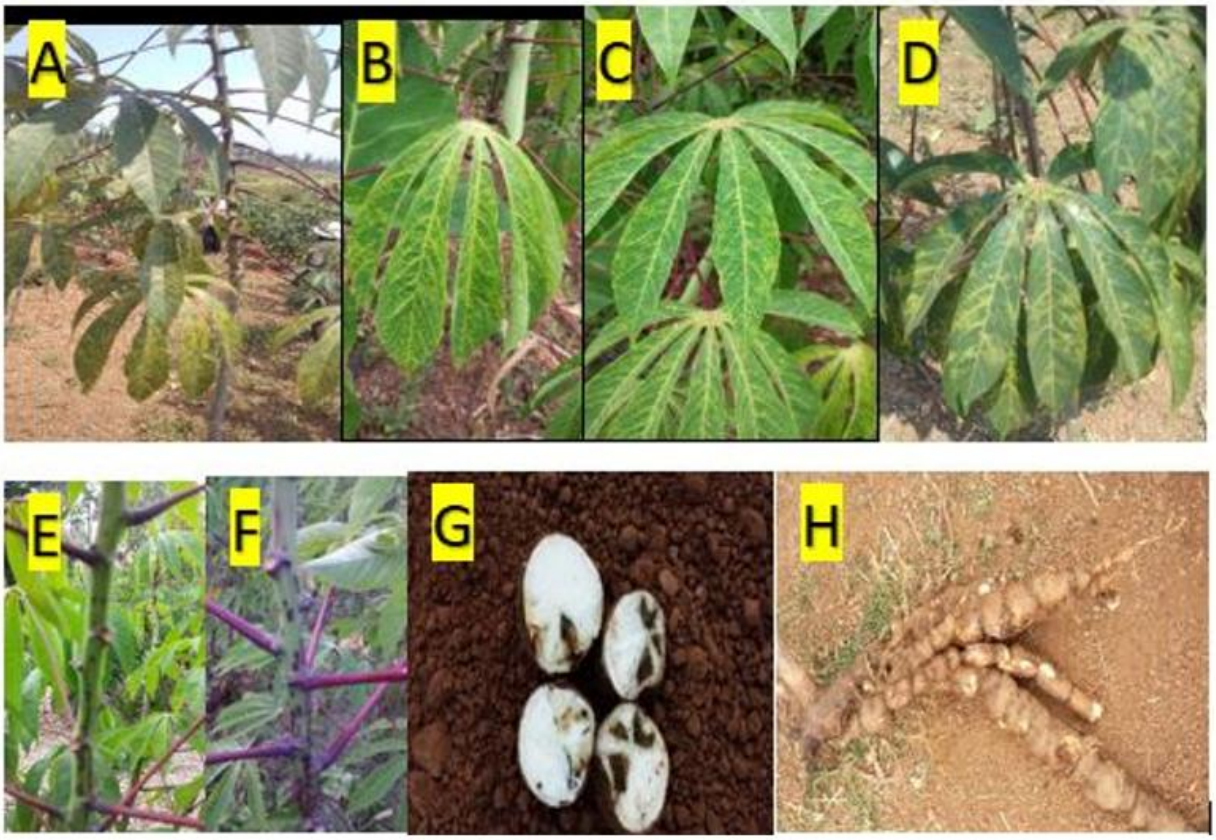

Fig. 1. Symptoms of cassava brown streak disease (CBSD) observed on cassava in Rwanda. (A) Interveinal chlorosis and chlorotic blotches. (B and C) Pronounced interveinal chlorosis along the secondary and tertiary veins. (D) Yellow patches, chlorotic spots. (E and F). Stems of CBSD-affected cassava plant expressing brown necrosis. . (G) Roots of CBSD-affected plant showing chalky brown necrosis. (H). Roots of CBSD-affected plant showing root constrictions.

CBSD severity

Overall, mean CBSD leaf symptom severity ranged from 2.0 to 2.8 on the cassava plants surveyed. In 2009, based on foliar disease symptoms, CBSD severity was high in Gisagara (3.2) and Nyagatare (2.9) districts. It was moderate in Huye (2.3), Gatsibo (2.4) and Bugesera (2.3) districts (Fig. 3 A).

In 2013, foliar disease severity was moderate in Bugesera (2.5) and Ruhango (2.2) districts. Based on CBSD root necrotic symptoms, CBSD severity was also moderate in Bugesera (2.5) and Gisagara (2.5) districts. It was low in Nyagatare (2.0), Kirehe (2.0) and Nyanza (2.0) districts (Fig.3 B).

In 2015, based on foliar disease symptoms, CBSD severity was highest in Nyamasheke (4.0) district, Western province and lowest in Nyagatare (2.0) district, Eastern province. Root necrotic severity was high in Kirehe (3.1), Kamonyi (3.0) and Nyanza (3.0) districts. It was low in Nyamasheke (2.0) district (Fig. 3 C).

In 2017, based on foliar disease symptoms, CBSD severity was high in Kamonyi (3.2), Kirehe (3.0) and Ruhango (3.0) districts. Root necrotic severity was highest in Gisagara (4.5) district, Southern province and lowest in Ruhango (2.0) and Nyagatare (2.) districts (Fig. 3 D).

Cassava brown streak viruses

Results of RT-PCR analysis showed the occurrence of both CBSV and UCBSV in Rwanda. This study showed that CBSV, UCBSV and co-infection were 10%, 9.6% and 1.7%, respectively. UCBSV was found in Southern, Eastern and Western provinces while CBSV occurred in Southern and Eastern provinces. The co-infections were found in Southern province (Nyanza and Gisagara districts) and Eastern province (Bugesera district). Results showed an increase of CBSV (from 0.9% to 10%) and UCBSV (from 2.8% to 9.6%) in 2013 and 2015, respectively. In 2017, the decrease in percentage for both

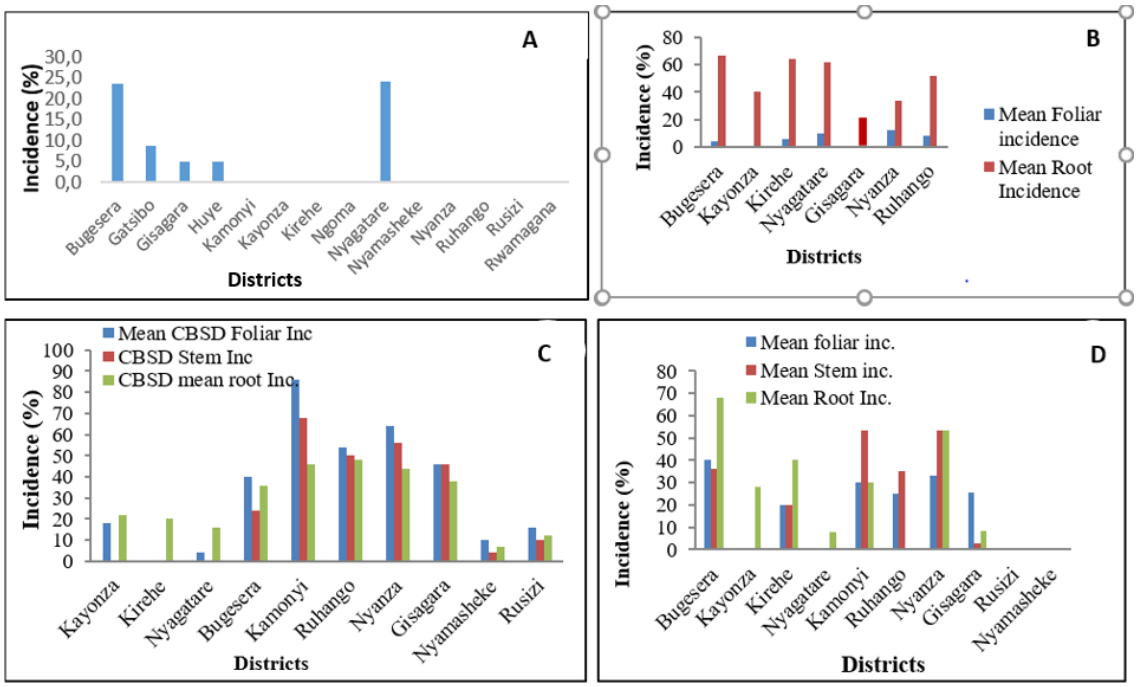

Figure 2. CBSD incidence (%) in Rwanda: A (year 2009), B (year 2013), C (year 2015) and D (year 2017).

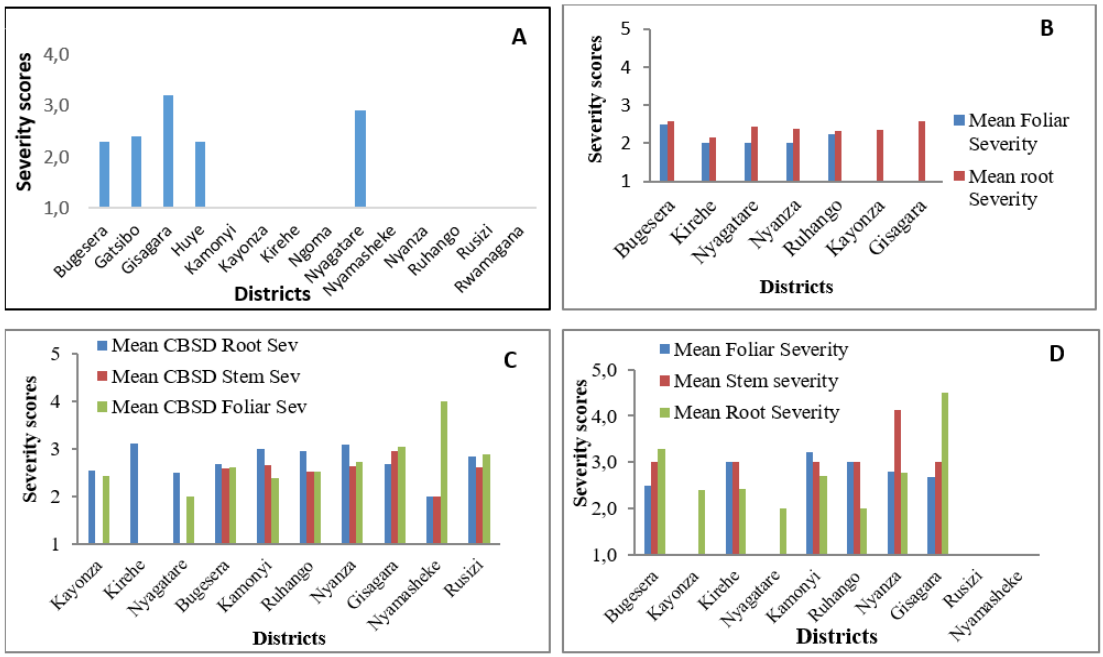

Figure 3. CBSD severity scores in Rwanda: A (year 2009), B (year 2013), C (year 2015), D (year 2017).

CBSV and UCBSV was observed compared to 2015 (Fig 4).

There was no association between virus species and symptom severity because both species, CBSV and UCBSV, were found on leaf samples which had severity scores of 2, 3 and 4. The same also for co-infection where in Bugesera district on genotype MH98/0105 with severity score 4 and in Nyanza district on genotype Ndamirabana with severity score 3.

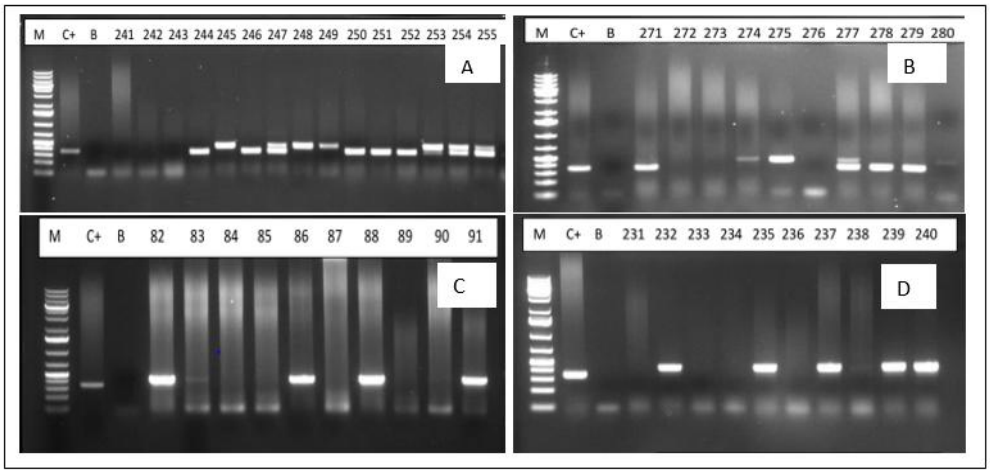

Figure 4. Gel electrophoresis of PCR products with Mbanzibwa primers CBSDDF2/R for CBSV (344 bp) and UCBSV (440 bp) detection in Rwanda: A (Nyanza), B (Gisagara), C (Kamonyi) and D (Rusizi).

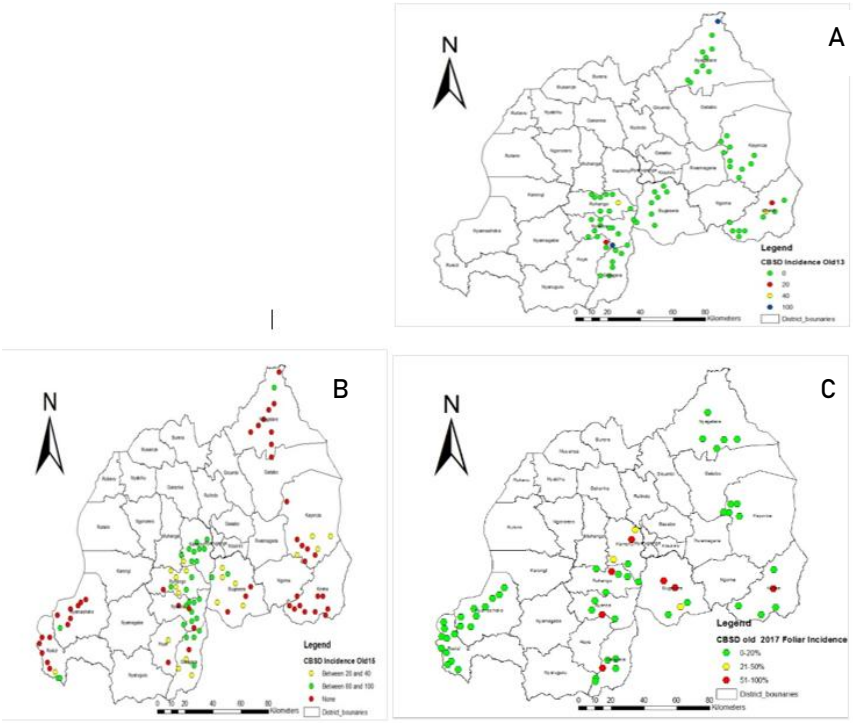

Fig 5. CBSD incidence distribution maps: A (2013), B (2015), C (2017) in Rwanda.

Geographical distribution of CBSD and associated viruses

In 2013, CBSD was distributed in Kirehe district, Eastern province and Ruhango, Nyanza and Gisagara districts of the Southern province (Fig. 5 A).

In 2015, Cassava brown streak disease was widely distributed in all cassava growing areas mainly in Kamonyi, Ruhango, Nyanza and Gisagara districts, Southern province; Bugesera, Kayonza, Nyagatare districts, Eastern province; and Nyamasheke and Rusizi districts, Western province (Fig. 5 B).

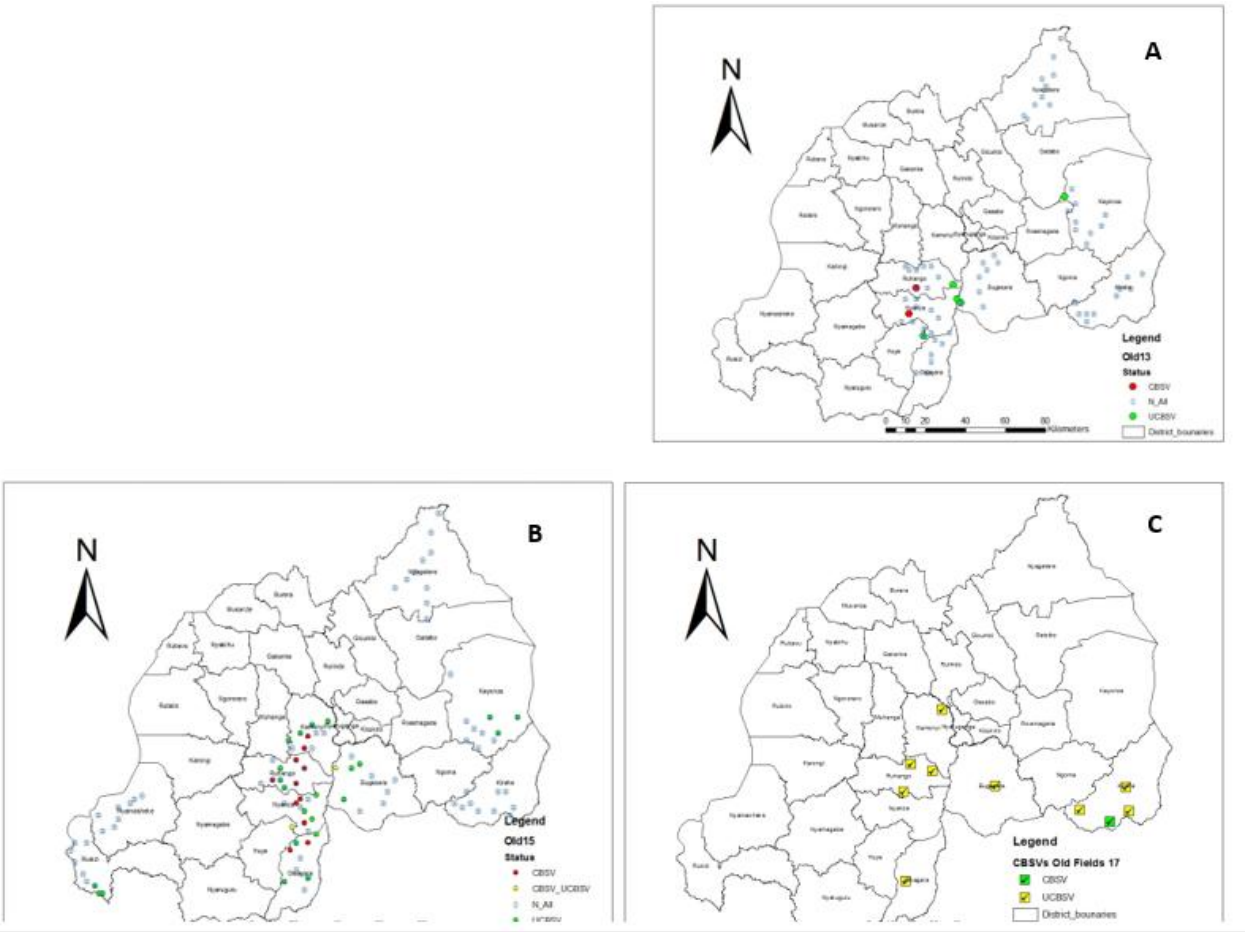

Fig 6. CBSVs distribution maps: A (2013), B (2015), C (2017) in Rwanda.

In 2017, CBSD was distributed, with low incidence, in Nyamasheke and Rusizi, Western province; while in Southern and Eastern provinces, it was distributed with high incidence, especially in Kamonyi, Ruhango, Nyanza and Gisagara districts in South; Bugesera and Kirehe districts in East (Fig. 5 C).

In southern, eastern and western provinces of Rwanda, UCBSV occurred widely while CBSV occurred in southern and eastern areas only (Fig. 6).

DISCUSSION

During field assessments over nine years (2009 to 2017), mean CBSD leaf incidence ranged from 5.8% to 37.1%. It was 13.2% in 2009, declined to the lowest record mean of 5.8 % in 2013, and increased to 37.1% and 28.8% in 2015 and 2017, respectively. The study showed that CBSD incidence was high in major cassava growing areas such as Kamonyi, Ruhango, Nyanza and Gisagara districts in Southern province; Bugesera, Kirehe and Nyagatare districts, Eastern province, while it was moderate in Rusizi and Nyamasheke districts, Western province.

Based on CBSD root necrotic symptoms, CBSD incidence was highest in Kamonyi (46%), Ruhango (48%), Nyanza (46%) and Gisagara (38%) districts, Southern province and Bugesera (36%), Kirehe (40%) districts, Eastern province. Overall, mean CBSD leaf symptoms severity ranged from 2.0 to 2.8 on cassava plants surveyed. Results showed that root necrotic severity was highest in Gisagara (4.5), high in Kamonyi (3.2) and Nyanza 93.0), Southern province; and Kirehe district (3.1) in Eastern province.

The current study confirms the presence of both CBSV and UCBSV in all cassava growing areas of Rwanda. Some unpublished reports previously reported that CBSV occurred only in Southern districts (Munganyinka *et al*., 2017). Current results confirm those of Munganyinka *et al*, (2017) reporting the presence of both CBSV and UCBSV in Rwanda. UCBSV was identified from cassava cultivars Ndamirabana and Mbakungahaze in Kayonza district located in Eastern Province. CBSV was identified on cassava cultivars Cyizere and Mbakungahaze in Kamonyi district, Southern Province. UCBSV was found in co-infection with CBSV from CBSD-affected cultivar Ndamirabana in Nyanza district, Southern Province. It was

observed that improved cassava varieties, introduced from the region, were more susceptible to CBSD (Ntawuruhunga and Legg, 2007; Mware *et al*., 2009). These improved CMD resistant varieties were introduced to control the CMD pandemic in Rwanda two decades ago (Night *et al*., 2011). These findings indicated that some local cultivars, for instance, Mushedile, Nyirakarasi, Gitamisi and Rutanihisha were less affected by CBSD. This sounds good as they can be incorporated in cassava breeding program in order to value their tolerance. The high CBSD incidence was observed in 2015 with 37.1% in average.

Results from previous studies in the region on cassava samples collected from Western and coastal Kenya, the main cassava regions of Uganda and inland Tanzania indicated that both viruses were found in all the three countries (Adams *et al.,* 2013). Those results found UCBSV to predominate in Uganda with a more even distribution between UCBSV and CBSV in the Lake Victoria region of Tanzania. Adams *et al.,* (2013) found UCBSV to predominate in the Kenya lowlands and Mbanzibwa *et al*., (2011) found CBSV to predominate in the Tanzania lowlands.

In 2011, cassava leaf samples collected in parts of Burundi bordering Lake Tanganyika revealed the widespread occurrence of CBSD-like symptoms. The diagnostic test from those samples provided the first confirmation that CBSD was present in Burundi and showed that UCBSV was so far the only virus species found in association with the disease in six provinces of Western and Southern Burundi (Bigirimana *et al*., 2011).

In 2016, cassava fields were inspected in Ituri Province, Northeastern DRC. Root necrosis and leaf symptoms consistent with CBSD were observed during field inspections in the two territories respectively on the Nyagota cultivar in Djegu, Mahagi Territory and Sawasawa cultivar in Alevu-Amaguna, Aru Territory. Laboratory results revealed mixed UCBSV + CBSV, UCBSV and no single CBSV infections. This first report confirmed the occurrence of both UCBSV and CBSV in the Northeastern part of DRC (Casinga *et al*., 2018). It is also reported that there might be more than two species of virus (Ndunguru *et al.,* 2015; Alicai *et al.,* 2016), and probably the current diagnostic primers cannot detect new species.

In Mayaga Agro-Ecological Zone including: Kamonyi, Ruhango, Nyanza and Gisagara districts, in Southern province and Bugesera district, in Eastern province, farmers used improved CMD tolerant cassava varieties like I92/0057 (Cyizere), MM96/0287 (Mavoka), MM96/3920 (Rwizihiza) and TME14 (Ndamirabana) which were unfortunately susceptible to CBSD. The spread and increase of CBSD incidence observed over nine years since CBSD-like symptoms were first observed in Rwanda in 2009 could be associated with the exchange and dissemination of CBSD-affected cuttings among farmers, especially with CMD tolerant varieties in all cassava growing areas in Southern and Eastern parts of Rwanda. Besides, Mayaga Agro-ecological zone, including Kamonyi, Ruhango, Nyanza and Gisagara districts in Southern province, is a high disease pressure compared to Eastern and Western Provinces.

The current study provides update about the occurrence and distribution of CBSD and associated viruses in Rwanda. The results of this study will inform all stakeholders working on cassava about the status of viral diseases and definitely contribute towards the enhancement of integrated viral disease management in cassava production areas.

CONCLUSIONS

Cassava brown streak disease is still the most important constraint for cassava production in Rwanda. Assessment of farmers' fields involved the scoring of leaf, stem and root symptoms of CBSD and the collection of leaf samples for virus identification and characterization using RT-PCR. The results showed that mean CBSD incidence varied by year and location. The disease occurred in Southern, Eastern and Western Provinces and was highest in Southern Province and lowest in Western Province. The current study confirmed the presence of both CBSV and UCBSV in all cassava growing areas of Rwanda. CBSV occurred only in Southern and Eastern provinces. These results confirmed also that UCBSV was more widespread than CBSV.

Much remains to be done, however, to generate more comprehensive molecular and biological information for these viruses. Deep sequencing of the total RNAs of viruses in samples from different districts of Rwanda could provide valuable additional information. Additional data of this type will strengthen our understanding of the probable origin and patterns of evolution of the CBSVs. Next generation sequencing technologies can have an important future role in identifying the causative agent(s) of CBSD-like symptoms in plants that test negative for CBSVs with existing diagnostics, as well as in identifying possible alternative hosts. The future work should also focus on identification, isolation and characterization of resistance genes from wild relatives of cassava, this will greatly help in achieving broad-spectrum resistance to CBSD.

RECOMMENDATIONS

Concerted efforts must be made quickly to develop and promote disease resistant cassava varieties with farmer-preferred attributes to accelerate adoption. Emphasizing the importance of distribution of healthy planting material to restrict the spread of the disease.

a) Short-term measures could explore the use of IPM options such as community phytosanitation. This management approach involves roguing out, in farmers' fields, the CBSD-affected plants at a community level.

b) This should be followed by supplying the farmers with clean (disease-free) planting materials, preferably of two or more cultivars.
c) In addition, awareness campaigns should be conducted to educate farmers, extensionists, NGOs and other stakeholders involved with the promotion of cassava about CBSD and its management.
d) Programs to monitor the disease should be made regularly and in consultation with the key stakeholders including scientists, cassava breeders, seed multipliers, plant health and crop protection services, NGOs, local governments and farmers.
e) Overall, for successful management of CBSD, major emphasis needs to be placed on mandatory virus indexing for germplasm exchange and surveillance of CBSD epidemics.

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