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Full Length Research Paper

Changes in the epidemiology of cassava mosaic disease and associated viruses in Rwanda: Occurrence and distribution since 2009-2017

Marie Claire Kanyange[1], Gervais Gashaka[1], Esperance Munganyinka[2], Marie Mutumwinka[1], Ghislain Niyonteze[1], Jeanine Umfuyisoni[1], Athanase Nduwumuremyi[1], Peter Sseruwagi[3], Fred Tairo[3] and Joseph Ndunguru[4]

[1]Rwanda Agriculture and Animal Resources Development Board, P.O. Box 5016, Kigali, Rwanda.
[2]National Council for Science and Technology, P.O Box 2285 Kigali, Rwanda.
[3]Mikocheni Agricultural Research Institute, P.O. Box 6226 Dar es Salaam, Tanzania.
[4]Tanzania Agricultural Research Institute Selian, P.O Box 6024, Arusha, Tanzania.

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Abstract

Cassava (*Manihot esculenta*) is an important food security crop in Rwanda. However, Cassava mosaic disease (CMD) is one of the major constraints to sustainable cassava productivity in the country. Four national surveys (2009, 2013, 2015 and 2017) were conducted to assess the continual prevalence of CMD and variations in incidences and severities in major cassava growing areas of Rwanda. The type of infections and virus species occurring were determined. The overall average mean CMD incidence was high (37.04%) in 2009 and low (5.4 %) in 2013. The incidences were less in 2015 and 2017 surveys compared to 2009 but higher than the ones recorded in 2013. The results showed that the cassava mosaic infections were generally associated with whitefly. Polymerase Chain Reaction (PCR) analysis showed occurrence of single infections of *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV) and co-infection with ACMV+EACMV. Single infections of EACMV were predominant (49.1% of total infections) in CMD-affected plants, followed by that of ACMV+EACMV (28.4%). Single infections of ACMV comprised of 22.5% of the total infections. This is the first study to report the occurrence and distribution of EACMV and co-infection of ACMV+EACMV based on molecular techniques in Rwanda.

Keywords: Incidence, Severity, Cassava mosaic disease, Whitefly.

LIST OF NON STANDARDS ABBREVIATIONS

ACMV	African Cassava Mosaic Virus
CMD	Cassava Mosaic Disease
CMBs	Cassava Mosaic Begomoviruses
DNA	Desoxyribonucleic Acid
EACMV	East African Cassava Mosaic Virus
EACMKV	East African Cassava Mosaic Kenya Virus
EACMMV	East African Cassava Mosaic Malawi Virus
EACMV-UG	East African Cassava Mosaic Virus Ugandan Variant
EACMZV	East African Cassava Mosaic Zanzibar Virus
GPS	Global Positioning System
ICMV	Indian Cassava Mosaic Virus

PCR	Polymerase Chain Reaction
SACMV	South African Cassava Mosaic Virus
SDS	Sodium Dodecyl Sulfate
SLCMV	Sri Lankan Cassava Mosaic Virus

INTRODUCTION

Cassava is the third most important source of calories in the tropics and more than 800 million people use cassava as a staple food crop and a source of income generation in Africa, Asia, and Latin America. Cassava is an important root crop in Rwanda with an annual production of 3,537,566 tons (FAOSTAT, 2016). It is under cultivation on 205,661 hectares with a yield average of 17.2 tons (FAOSTAT, 2018). The crop is grown across the country and is a staple food crop for 12,501,156 people (UN Data 2018). Production of this crop is constrained by different factors including mainly pests and diseases among others. Viruses present the biggest disease threat to cassava productivity in Africa. Cassava mosaic disease (CMD) is endemic throughout sub-Sahara Africa and wherever cassava is grown (Chikoti et al., 2019; Mallowa et al., 2006; Legg et al*.,* 2005; Were et al*.,* 2004). Cassava mosaic disease is caused by cassava mosaic begomoviruses (CMBs) (Genus *Begomovirus*; family *Geminiviridae).*

Studies have identified different *Begomovirus* species in association with CMD in different regions of Africa including *cassava mosaic virus* (ACMV), *East African cassava mosaic virus (*EACMV), *East African cassava mosaic Cameroon virus* (EACMCV) (Fondong, et al., 2000), *East African cassava mosaic Kenya virus* (EACMKV) (Bull et al., 2006), *East African cassava mosaic Malawi virus* (EACMMV) (Zhou et al., 1998), *East African cassava mosaic Zanzibar virus* (EACMZV) (Maruthi et al., 2004), and *South African cassava mosaic* (SACMV) (Berrie et al., 1998). Two other viruses, *Indian cassava mosaic virus* (ICMV) (Matthew et al., 1992, Saunders et al., 2002) and *Sri Lankan cassava mosaic virus* (SLCMV) (Saunders et al., 2002), were reported from the Indian sub-continent.

The different virus species that cause the disease are transmitted rapidly by the whitefly (*Bemisia tabaci*). Since cassava is propagated by use of stem cuttings, the inadvertent use of virus-infected planting material further contributes to dissemination of CMD. CMD produces a variety of foliar symptoms that include mosaic, mottling, misshapen and twisted leaflets, and an overall reduction of root size and a general decline of the plant. CMD alone causes an estimated 47% yield loss equivalent to more than 13 million tons annually in East and Central Africa (Legg *et al.,* 2006). Previously, CMBs were thought to show geographic structuring with ACMV limited to West and Central African countries towards the west of the Rift Valley and in South Africa, EACMV to the eastern part of the Rift Valley in the coastal Kenya, Tanzania, Malawi, Zimbabwe and Madagascar, and ICMV to India and Sri Lanka (Harrison et al., 1997). However, subsequent reports have shown that most of the seven CMBs reported from sub-Saharan Africa are widespread across the sub-continent (Atiri et al., 2004, Berry et al., 2001, Bull et al., 2006, Patil et al., 2009), whereas ICMV and SLCMV appear to have remained confined to cassava-growing regions of India and Sri Lanka (Patil et al., 2009, Thottappilly et al., 2003).

Cassava mosaic disease has become more important in recent years following the spread of the regional pandemic of severe mosaic disease from Uganda to neighbouring countries including Rwanda (Legg et al., 2006). Comprehensive surveys of cassava mosaic disease in Rwanda were conducted in 2001, 2004 and 2007 in the major cassava growing areas of Southern and Eastern Rwanda (Sseruwagi et al., 2005; Gashaka et al., 2007; Night et al., 2011) to study the incidences and severity of cassava mosaic disease. Furthermore, ACMV and EACMV-UG were reported as virus species involved in CMD in Rwanda. Through these surveys, the spread into Rwanda of the severe cassava mosaic virus disease pandemic was reported. There is a need to understand of what is associated to this occurrence of severe cassava mosaic disease in Rwanda. The current study aimed (i) to study and monitor changes in the incidence and severity of CMD across the country and over a period of eight years, (ii) to understand the source of CMD infection (vector-borne or cutting-borne), and (iii) to determine the viruses associated with the disease and their distribution.

METHODOLOGY

Field assessment of incidence and severity of CMD

Field surveys were conducted in 2009, 2013, 2015 and 2017 to study and monitor the changes in the incidence and severity of CMD. A total of 14 districts (Bugesera, Kayonza, Nyagatare, Rwamagana, Gatsibo, Ngoma and Kirehe, Rusizi and Nyamasheke, Gisagara, Huye, Nyanza, Ruhango and Kamonyi), in 2009; 7 districts (Bugesera, Kayonza, Nyagatare, Kirehe, Gisagara, Nyanza and Ruhango), in 2013 and 10 districts (Bugesera, Kayonza, Nyagatare, Kirehe, Rusizi, Nyamasheke, Gisagara, Nyanza, Ruhango and Kamonyi) in 2015 and 2017 were surveyed. Cassava fields belonging to 89 farmers (year 2009), 67 farmers (year 2013),

100 farmers (year 2015) and 100 farmers (year 2017) were examined for CMD presence. Sampling distance between two fields was at least 8 km. The altitude, latitude and longitude of each field were recorded using Global Positioning System equipment (GPS). Thirty plants were examined for the presence or absence of CMD symptom along the two diagonal transects of the field to determine incidence and symptom severity of the disease. The incidence of CMD was calculated from the number of plants with disease symptoms expressed as a percentage of the total plants assessed in the field (Sseruwagi *et al*., 2004; Fauquet and Fargette 1990). CMD symptom severity was assessed using 1 to 5 scoring scale of Hahn et al. (1980), where 1 represents symptom-free plants and 5 severely diseased plants. In calculating mean severity per field, scores of '1' (no visible symptoms) were excluded. This allowed for a true evaluation of the degree of damage caused by CMD on the affected plants.

Assessment of infection type and whitefly population

Infection types were categorized as "C", "W" and "H" for cutting, whitefly-borne infections and healthy, respectively. Where the lower first formed leaves showed symptoms, infection was assumed to be cutting-borne, while where only upper leaves were symptomatic, infection was considered whitefly-borne. Adult whitefly (*Bemisia tabaci*) was counted on the top five fully expanded apical leaves for the tallest shoot of each of the 30 plants assessed per field and the total recorded.

Sampling of test materials

Leaf samples from plants with characteristic CMD symptoms (mild to very severe symptoms) were sampled for viral testing using molecular techniques. Three young cassava leaves were collected from each field and a total of 267 (year 2009), 201 (year 2013), 300 (year 2015), 300 (year 2017) samples were collected.

Extraction of total nucleic acids

Total DNA was extracted from the collected leaf samples using the protocol of Dellaporta as modified by Ndunguru, 2007. Briefly 100mg of plant leaf tissue was ground in 700µl of Dellaporta extraction buffer, pre-warmed to 65°C using mortar and pestles (which were sterile and autoclaved) until uniform mixture. The mixture was transferred into 1.5ml Eppendorf tube and added 45µl of 20% sodium dodecyl sulphate (SDS) to the tube. The mixture was incubated in water bath at 65°C for 30 min, then 200µl of 3M sodium acetate (pH 5.2) were added to each tube and mixed thoroughly by vortexing. The tubes were incubated on ice for 20min and centrifuged at 13000 rpm for 10min at room temperature in a microfuge. Then, 500µl of the supernatant were transferred to new Eppendorf tube avoiding leaf debris. In a fume hood, equal volume of 500µl of chloroform: iso-amyl (24:1) was added and mixed gently by inverting the tube, centrifuged at 13000 rpm for 10min and the upper aqueous layer (approximately 400-450) was transferred into a new 1.5ml Eppendorf tubes. An equal volume of cold isopropanol (pre-chilled in -20 °C freezer) was added, mixed thoroughly by gently inverting the tube, then incubated at -20°C for 30min and centrifuged to precipitate the DNA. The DNA pellets obtained were washed in 500 µL of 70% ethanol and suspended in nuclease free water.

Virus detection by PCR

The extracted DNA was stored at -20°C before PCR test. The primers JSP001/JSP002 (Universal primers) were used for detection of ACMV (coat protein) and EAB555/F/EAB555/R degenerate primers were used for detection of EACMV DNA B. The reaction mixtures contained 2.5µl of the PCR reaction buffer 10x, 2.5µl MgCl2, 0.5µl dNTPs, 1µl of each forward and reverse primer, 0.5µl of Taq DNA polymerase and 2µl of DNA sample. Amplification conditions included a first PCR cycle comprising denaturation at 94°C for 2 min, annealing at 58°C for 1 min and elongation at 72°C for 2 min. The initial amplification cycle was followed by 35 cycles of 1 min at 94°C, 1 min at 58°C and 2 min at 72°C. At the end of the reaction, a final elongation was performed at 72°C for 10 min. The PCR products were separated by agarose gel electrophoresis on a 1% (w/v) gel in TAE buffer. The DNA bands were visualized following ethidium bromide staining under UV light and photo were taken using gel documentation system.

RESULTS

Cassava mosaic disease Incidence and severity of symptoms

High incidence of CMD was recorded in the districts surveyed in 2009 with the overall average mean incidence of 37.04%. The low average mean incidence (5.4 %) was recorded during the survey conducted in 2013. The average mean incidence of 20.18% and 24.37% were recorded in 2015 and 2017 surveys, respectively. The 2009 survey revealed low incidence in Kamonyi (13, 3%) and the highest in Huye (>80%) districts of southern province. In the 2013 survey, the incidence was significantly low in the surveyed districts (Figure 1) with the highest incidence (20.7%) recorded in Nyagatare district of eastern province and the low incidence (<5%) recorded in Ruhango district of southern province. There was an increase of CMD incidence during 2015 and 2017 surveys compared to 2013 survey. During the surveys of 2015 and 2017 the high incidence was recorded in the districts of eastern province (Kayonza, Kirehe and Nyagatare)

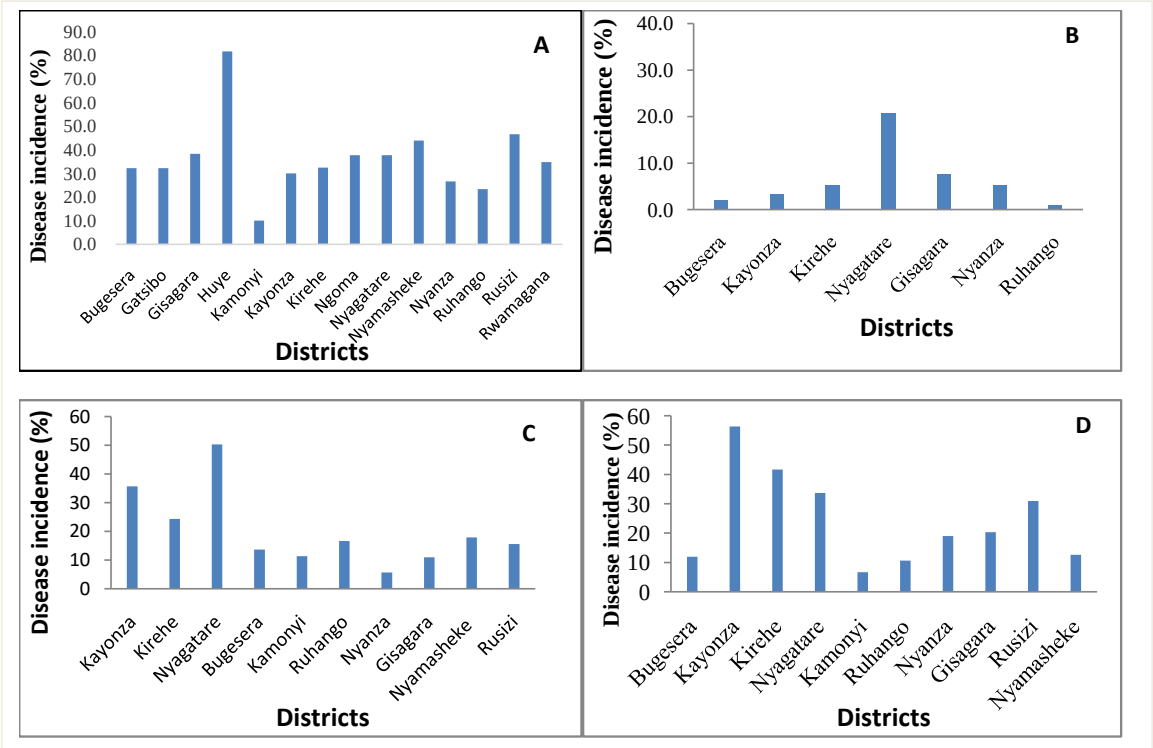

Figure 1. Incidence of cassava mosaic disease in cassava growing districts of Rwanda A (2009), B (2013), C (2015) and D(2017).

whereas the low incidence was observed in the districts of southern province (Kamonyi, Ruhango and Nyanza) (Figure 1).

Cassava mosaic disease symptom severity scores varied little from different surveys within districts. The scores ranged from 2.8 to 4.3 in 2009 survey, from 2.8 to 3.4 in 2013 survey, from 2.4 to 3.5 in 2015 survey and from 3.0 to 4.0 in 2017 (Figure 2).

Source of CMD infection

The type of CMD infection was assessed during surveys of 2015 and of 2017. Data presented in table 1 indicate that whitefly borne infection (13.2% in 2015 and 14.0 % in 2017) was high compared to cuttings borne infection (6.9 % in 2015 and 10.4 % in 2017) in both surveys. Of special note, infections observed in districts of western province (Nyamasheke and Rusizi) in 2015 were only associated with cuttings. In general, the frequency of whitefly infection was typically very low in the western districts and high in eastern districts.

Abundance of whitefly

The whitefly abundance was investigated, and the results presented in figure 3 indicate that the whitefly counts were less (≤ 20 adult whitefly/plant) during 2009 surveys. However, significantly higher whitefly abundance (≥ 200 adult whitefly/plant) were observed in 2013 and 2015 surveys. There was a decrease in whitefly density during the survey conducted in 2017. Across all surveys, Bugesera district recorded higher density of whitefly whereas Rusizi and Nyamasheke recorded less densities of whitefly.

Viruses associated with CMD and their distribution

The viruses associated with CMD were investigated using molecular techniques by PCR (Figure 4). The findings revealed that CMBs were widely distributed in Rwanda. In 2009, 271 out of 283 leaf samples analyzed gave positive results, of which 258 (91%), 93 (32, 8%) and 80 (28, 2%) samples were infected with EACMV, ACMV and ACMV+EACMV, respectively. A total of 12 (4.2%) samples were negative to all viruses tested using the available primers. The results on the occurrence of virus species indicated that 1.18%, 8.96% and 0.05% of the samples had infection of EACMV, whereas 0%, 2.15% and 0.01% had ACMV in 2013, 2015 and 2017, respectively. There were low percentage (1.07%) of co-infections of ACMV and EACMV recorded in affected fields during the 2015 survey. No co-infection was recorded in surveys conducted in 2013 and 2017.
The distribution map (Figure 5) of CMBs showed the occurrence of one strain (EACMV) in all districts surveyed

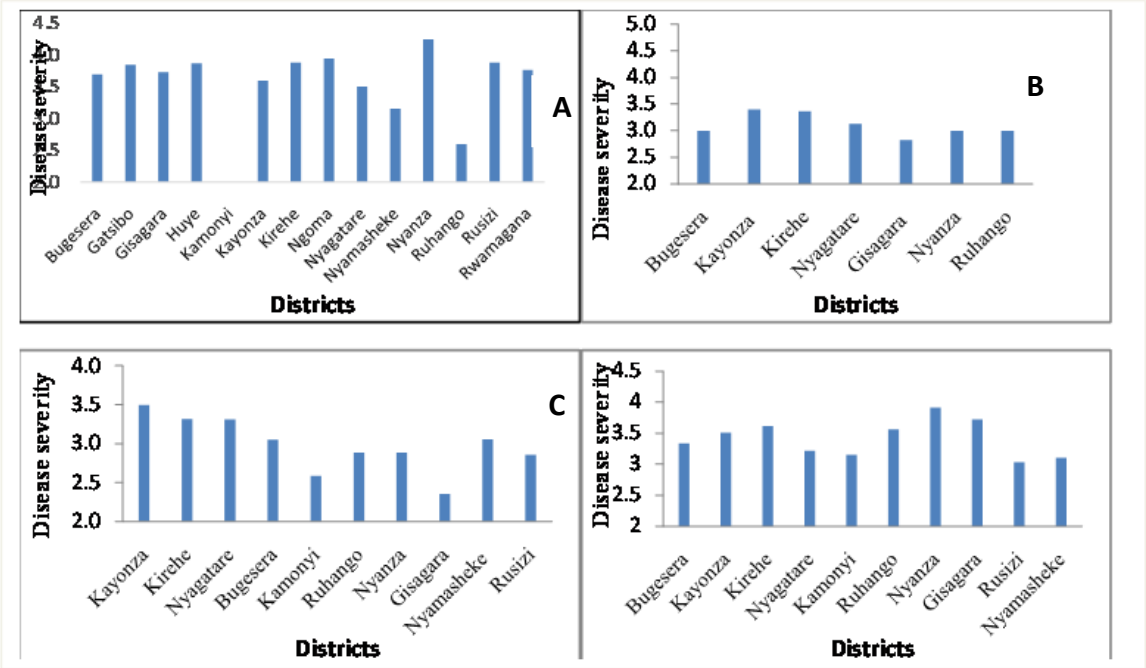

Figure 2. Severity of cassava mosaic disease (CMD) in Rwanda A (2009), B(2013), C(2015), D(2017).

Table 1. Source of cassava mosaic disease (CMD) infection in relation to districts surveyed in 2015 and 2017 surveys in Rwanda.

Districts	CMD incidence (%)		Frequency of source of infection (%)			
			Survey 2015		Survey 2017	
	2015	2017	Cutting	Whitefly	Cutting	Whitefly
Eastern province						
Kayonza	35.6	56.3	5.3	30.3	15.3	41.0
Kirehe	24.2	41.6	6.0	18.2	13.5	28.1
Nyagatare	50.3	33.6	8.7	41.6	22.0	11.6
Bugesera	13.6	11.9	2.3	11.3	2.3	9.6
Southern province						
Kamonyi	11.3	6.6	1.0	10.3	1.0	5.6
Ruhango	16.6	10.6	3.6	13.0	2.0	8.6
Nyanza	5.6	19.0	2.6	3.0	13.0	6.0
Gisagara	10.9	20.2	6.3	4.6	4.6	15.6
Western province						
Nyamasheke	17.8	12.6	17.8	0.0	8.3	4.3
Rusizi	15.3	30.9	15.3	0.0	21.6	9.3

in 2013 except Ruhango and Kayonza districts. During the 2015 survey, two strains (EACMV, ACMV) were detected in different districts with EACMV present in all districts except Nyanza, Rusizi and Nyamasheke. ACMV was present in Nyanza and Nyamasheke districts. Furthermore, two strains (ACMV and EACMV) were detected in 2017 with ACMV present in Gisagara district only and EACMV in Bugesera, Kayonza, Kirehe, Nyagatare and Nyanza districts.

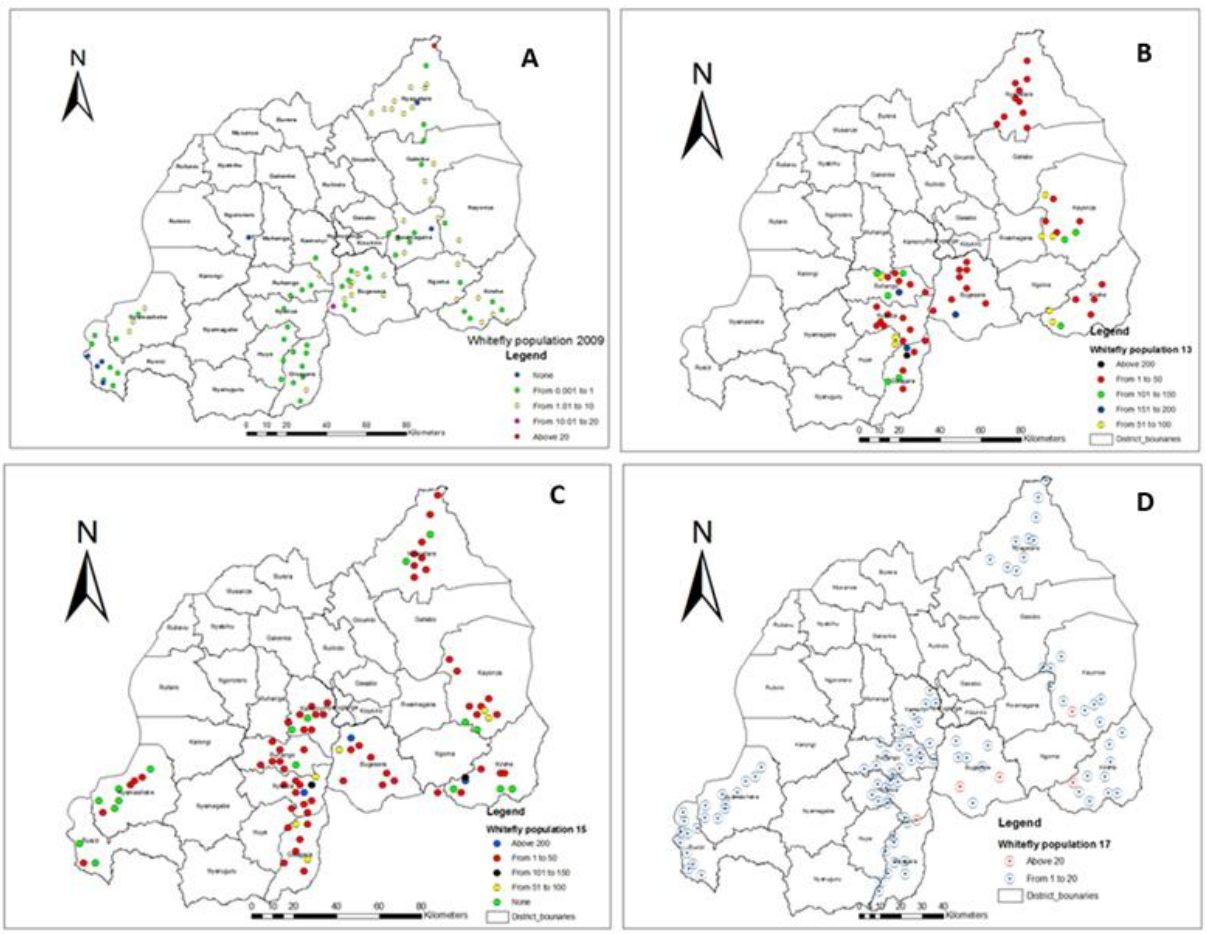

Figure 3. Populations of whitefly in Rwanda A (2009), B (2013), C (2015) and D (2017).

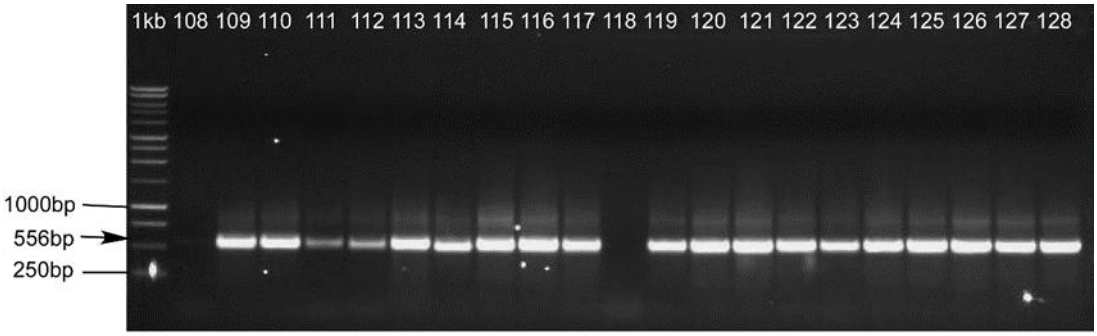

Figure 4. Molecular detection of EACMV. Lanes 108 to 128 exhibited the amplification of EACMV using EAB555/F (5'-TACATCGGCCTTTGAGTCGCATGG-3') and EAB555/R (5'-CTTATTAACGCCTATATAAACACC-3').

DISCUSSION

Cassava mosaic disease occurs in all the cassava-growing areas of Africa and is the most important disease of cassava (Geddes, 1990) on this continent. The epidemic situation, as encountered in the 1990s in much of Uganda, spread to adjacent areas of western Kenya and north-west Tanzania and later into Rwanda and Burundi (Legg & Thresh, 2000; Otim-Nape et al., 2000; Legg et al., 2001; Bigirimana et al., 2004; Sseruwagi et al., 2005). In 2000, a first survey on CMD was conducted in five regions of Rwanda and reported the spread of the severe cassava mosaic virus disease pandemic with disease incidence of 20% (Legg et al., 2001). Not later

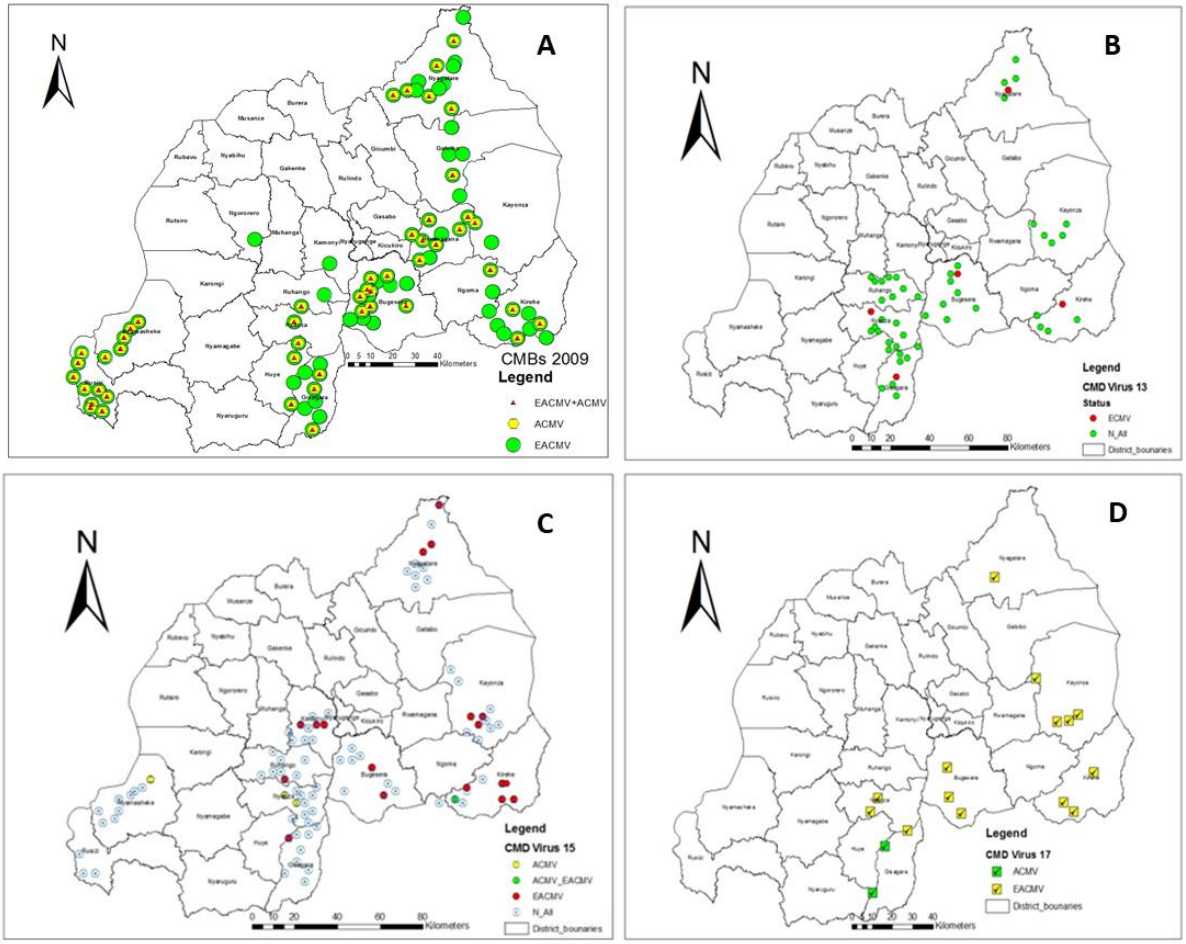

Figure 5. Occurrence and distribution of CMBs in Rwanda A (2009), B(2013), C(2015) and D (2017).

than one year, a countrywide survey was carried out and reported an increase in CMD incidence (30%) (Sseruwagi, 2001, unpublished). Since then, there was a considerable information on the incidence and severity of CMD in the country (Sseruwagi et al., 2005; Gashaka et al., 2007; Night et al., 2011). However, farmers continued to experience serious losses that food security was threatened. This study was designed to monitor the disease over eight years and to identify the main source of spread of infection and the viruses causing the disease. This is the first study to report the occurrence and distribution of EACMV and co-infection of ACMV+EACMV based on molecular techniques in Rwanda.

This study reports the continual prevalence of CMD in Rwanda. The CMD incidences were high in 2009 (37.04%) compared to 2013 (5.4%), 2015 (20.18%) and 2017 (24. 37%). The considerable progress in the control of CMD in Africa was from breeding efforts and the identification of resistance traits that conferred a high level of protection against all viruses associated with the disease (Fondong, 2017; Lokko *et al.,* 2009; Dixon and Ssemakula, 2008). The improved CMD-resistant cultivars in Rwanda were introduced from neighbouring Uganda and subsequently propagated and distributed to farmers. The CMD incidence was low in districts of southern province where farmers grow more of the improved CMD-resistant cassava varieties. However, the majority of farmers (83%) grew only local cultivars and a limited number (7.5%) grew only improved cultivars and the rest applied the mixture of local and improved cultivar (9.5%) (data not shown). This has contributed to the sustainability of the disease in the country and there is a need for the increased use of CMD-resistant varieties through breeding programmes or selected by farmers from those already available and being grown. Given farmers' preference for local cassava cultivars, introducing cleaning planting materials using tissue-culture-based techniques would be a good approach to implement in cassava seed systems in the country. Based on the survey results from the current study, CMD infection was associated mainly with whitefly. There was large increase in vector populations over the years. The populations of whitefly increased 10 times from 2009 to 2015.

Table 1. Source of cassava mosaic disease (CMD) infection in relation to districts surveyed in 2015 and 2017 surveys in Rwanda.

Districts	CMD incidence (%)		Frequency of source of infection (%)			
			Survey 2015		Survey 2017	
	2015	2017	Cutting	Whitefly	Cutting	Whitefly
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Southern province						
Kamonyi	11.3	6.6	1.0	10.3	1.0	5.6
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Nyanza	5.6	19.0	2.6	3.0	13.0	6.0
Gisagara	10.9	20.2	6.3	4.6	4.6	15.6
Western province						
Nyamasheke	17.8	12.6	17.8	0.0	8.3	4.3
Rusizi	15.3	30.9	15.3	0.0	21.6	9.3

However, in 2017 the whitefly density decreased 10 times less. These fluctuations were some of the indicators of the changes in the epidemiology of CMD observed during this study. CMD is being spread rapidly by the whitefly vector (*B. tabaci*) and the symptoms of the disease are usually prevalent and severe (Thresh and Cooter, 2005). The findings from the current study indicate no CMD infection due to whitefly in Nyamasheke and Rusizi in 2015. In 2017, few plants showed infections caused by whitefly. These results explore that new plantings were colonized by immigrant whiteflies moving from older stands of cassava in the area. In order to reduce both disease incidence and the risk of more virulent isolates emerging, the control of viruses as well as vectors is therefore a key topic. It is therefore recommended to screening Rwandan cassava germplasm to identify the genetic base for resistance to CMD and whitefly.

The current study reported the occurrence of different viruses or virus combinations associated with CMD in different regions of Rwanda. The begomovirus species of ACMV and EACMV and a co-infection with the two-virus species were identified. Previous studies reported the occurrence of Uganda variant of East African cassava mosaic virus (EACMV-UG) in Rwanda (Legg et al., 2001). The presence of the new virus species and strains could complicate and may even undermine the effectiveness of resistance cassava breeding programmes in the country. This implies the need for a periodic disease monitoring and surveillance. In addition, empowering the farming community (know-how transfer) to grow healthy, nutritious cassava would contribute in the management of CMD diseases.

CONCLUSION

The data generated in this study will increase knowledge of CMD epidemiology in Rwanda. Furthermore, the information provided in this work is a good foundation for future work and can be used as a base on which to formulate a sustainable CMD control and management strategies in Rwanda. The measures could include, use of uninfected propagules for all new plantings and avoid moving infected cassava plants or vegetative propagules between different districts or regions and especially from areas affected by a severe form of CMD. This may lead to greater production of cassava in the country given the need to increase food production to feed the increasing human population

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