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# Incidence and characterization of cassava mosaic viruses in improved and local cultivars in Sierra Leone

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**In Sub-Sahara Africa, Cassava Mosaic Disease (CMD) is the major important viral disease of cassava which threatens production, livelihoods and food security. Frequently CMD is due to the Africa Cassava Mosaic Virus (ACMV), East African Cassava Mosaic Virus (EACMV) and the Ugandan strain of the East African virus (EACMV-UG). The present study investigated the prevalence of these three viruses in 27 cultivars (three improved and 24 local) of cassava grown across all key agro-ecological zones and regions in Sierra Leone. Leaf samples from each of these varieties with symptoms as well as asymptomatic ones were harvested from a clonal garden established on the Njala University Campus. Deoxyribonucleic acid (DNA) extracted from these samples was assessed with Marker Assisted Selection (MAS) for ACMV, EACMV and EACMV-UG using polymerase chain reaction (PCR) and primers designed to amplify replica regions of the DNA-A components of these viruses. The results revealed the ACMV is very common (26 out of 27 samples), while EACMV and EACMV-UG are less common (3 out of 27). Samples with EACMV-UG also had ACMV. These findings are of importance to future breeding programs for resistance to CMD with the aim of producing multiple viral resistant clones for increased food security in the country.**

**Key words:** Cassava, DNA, characterization, mosaic viruses, incidence, varieties, Sierra Leone.

## INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a significant source of carbohydrate for millions of people across the world (Mbanzibwa et al., 2011; Ntui et al., 2015). It is also a major source of raw materials for industrial processes like starch and biofuels (Jasson et al., 2009). Approximately half of the population in Africa produces cassava (Nweke, 2004). Some 8.5 metric tons of cassava are exported yearly and this shows how important the production of the crop is in the world economy (Cooper et al., 2015). The key advantage of cassava is that it grows and survives on nutrient depleted soil and once established is drought resistant (Parmar et al., 2017; Nweke, 2004; Thresh et al., 1994). Despite its importance for food and income in many developing countries, production is often constrained by biotic factors like Cassava Mosaic Disease (CMD) and insect pests (Campo et al., 2011; Waddington et al., 2009).

The fresh leaves of cassava contain high levels of protein (17-18%) and constitute an important source of leafy green vegetable for the majority of households in

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Sierra Leone (Aduni et al., 2005). It is the second most important food crop in Sierra Leone after rice, but the average yield of cassava is low, and has been estimated at 7.18 metric tonnes/ha (FAOSTAT, 2015) compared to 8.4 metric tonnes/ha for Africa. Despite its tolerance of drought conditions and other environmental factors, low yields can arise due to disease and pest, weed, changing climatic conditions and selection preferences for sweet varieties associated with low cyanide content by farmers and consumers (Houngue et al*.,* 2018; Berry, 2001).

Financial losses due to disease are huge and rising across the continent; and estimates made some two decades ago put the loss at billions of dollars (Thresh et al., 1997). The major disease affecting cassava is CMD caused by several viruses that belong to the genus *Begomovirus* of the family *Geminiviridae* (Bull et al., 2006). Eight out of seventeen of these viruses that affect cassava are endemic to Africa (Hillocks et al., 2000; Thresh et al., 1994; Bull et al., 2006). CMD is a major constraint to cassava production in Africa (Legg et al., 2006).

Some of the viruses have bipartite genomes termed DNA-A and DNA-B (Stanley et al., 2004). In a review, Fauguet et al. (2008) noted some distinct but similar viruses: Africa Cassava Mosaic virus (ACMV), East Africa Cassava Mosaic virus (EACMV), East Africa Cassava Mosaic Ugandan Virus (EACMV-UG), East Africa Cassava Mosaic Cameroon Virus (EACM-CV), East Africa Cassava Mosaic Kenya Virus (EACM-KV), East Africa Cassava Mosaic Malawi Virus (EACM-MV), East Africa Cassava Mosaic Zanzibar Virus (EACM-ZV) and South Africa Cassava Mosaic Virus (SA-CMV). A recombinant begomovirus novel called EACMV-UG was associated with a severe outbreak of CMD in Uganda (Deng et al., 1997; Zhou et al., 1997); which now appears to be spreading to neighboring countries and beyond. Previous studies indicated that the virus can be found either on its own or mixed with ACMV (Ntawuruhunga et al., 2007; Harrison et al., 1997; Pita et al., 2001 Briddon, 1995). EACMV-UG genome can be distinguished from the EAC-MV strain by the presence of a region in the coat protein AV1 gene of approximately 500 base pairs with high identity sequence to the corresponding part of the ACMV genome.

Scientists in Africa have drawn attention to the types of viruses causing cassava mosaic disease and cassava brown streak diseases, emphasizing the inadequate attention given to the significant losses caused by these viruses to African farmers (Hilocks et al., 2000; Nwokoro 2005). Among the four countries that comprise the Mano River Union (Cote d'Ivoire, Guinea, Liberia and Sierra Leone), Sierra Leone is the largest producer of cassava, but production is by a very large number of small-holder farms. The success of cassava production will influence the country's food security; production has a negative correlation with CMD presence. Many local cultivars are already infected with CMD and the disease is widespread across the country.

Samura et al. (2016) and Sesay et al. (2016) reported using species-specific PCR to identify the viruses associated with CMD in six plants from southern Sierra Leone; they showed the presence of both ACMV and EACM. Despite these studies, little data exist on molecular characterization and genomic variability of these viruses across the country, and this is critical in the development of control strategies. The present study was designed to detect the incidence of CMD and characterize the different strains of CMV associated with the known cultivars of cassava across the key agro-ecological zones of Sierra Leone.

## MATERIALS AND METHODS

### Field plot establishment

A total of twenty-seven varieties (Table 3) were established on a plot at the Biological Sciences Experimental Farm, Njala University (08° 14' S, 12° 1' W). The experiment was laid out in a randomized complete block design with three replicates of 23 local and 4 "improved" cultivars. The planting materials comprised stem cuttings collected from all four agro-ecological zones namely; rainforest, savannah, grassland and mangrove (Table 1), planted with a planting distance of 1m × 1m between and within rows. Twenty-centimetre long cuttings were obtained and planted in February and watering continued weekly until the rains became consistent in May.

### In-field assessment of pest and disease

The leaves were visually assessed for symptoms of CMD and for the presence of the "tobacco" whitefly (*Bemisa tabaci* var. *gennadius*) during the early morning hours. Incidence and severity of the cassava mosaic virus were assessed at one and three months after planting using a 1-5 disease rating scale adopted by Legg (1999) (Table 1).

### Molecular diagnosis and quantification

Samples from young leaves of the 27 cassava cultivars were collected and dried using silica gel for 10 days in "zip-lock" bags. DNA analysis and molecular diagnostics was undertaken at the National Crops Resources Research Institute (NaCRRI), Biosciences Laboratory, Nalounge, Kampala, Uganda.

### DNA extraction and quantification

The extraction of plant DNA was done using the CTAB protocol with some modifications (Doyle et al*.,* 1990). Dried leaf samples weighing approximately 0.2 g were ground with sterile beads in 2-ml Eppendorf tubes in 500 µl of extraction buffer (0.1M Tris-HCL with pH 8.0; 0.02 M Ethylene Diamine Tetra-acetic Acid (EDTA); 0.5 M HCl; 1% polyvinylpyrolidone and 10% CTAB). An addition of 50 µl of 10% CTAB was made and mixed by inversion, with the tubes incubated for 30 min at 65°C. Exactly 250 µl of chloroform:isoamyl alcohol was added next at -20°C and shaken by inversion for 10 min to allow mixing and followed by spinning for 10 min.

**Table 1.** Disease scoring scale utilized for in-field disease assessment.

| Scale | Chlorotic | Leaf distortion | Stunting |
|---|---|---|---|
| 1 | None | None | None |
| 2 | Mild | Base | None |
| 3 | Pronounced | Lower 1/3 | None |
| 4 | Severe | Lower 2/3 | Some |
| 5 | Severe | Whole leaf | Severe |

**Table 2.** PCR amplification using species-specific primers.

| Species-specific primers | Forward primer sequences | References |
|---|---|---|
| Primers (ACMV-AL1/F) | 5'-GCGGAATCCCTAACATTATC-3' | Mills et al. (1992) |
| Primers (ACMV-ARO/R) | 5'-GCTCGTATGTATCCTCTAAGGCCTG-3' | Mills et al. (1992) |
| Primers (UV-AL1/F1) | 5'-TGTCTTCTGGGACTTGTGTG-3' | Mills et al. (1992) |
| Primers (ACMV-CP/R3) | 5'-TGCCTCCTGATGATTATATGTC-3' | Mills et al. (1992) |

Centrifugation of samples was done for 10 min, and 500 µl of Isopropanol (-20°C) was added to the supernatant in a new tube. The mixture was incubated for 5 min at -20°C, centrifuged and the supernatant discarded. The washing of pellets was done once with 700 µl of 70% ethanol at -20°C and pellet air-dried. The pellets were then re-suspended in 150 µl of 10:1 TE (10 mMTris:1 mM EDTA) buffer. A 2.25 µl of 10 mg/Ml RNase was added to the suspension and incubated for 5 min at 65°C, and the DNA stored at -20°C until needed.

The extracted DNA was quantified using a Nanodrop ND-1000 spectrophotometer (Thermo fisher Scientific Inc.; Wilmington, DE, USA), to check the quality and purity of the DNA. The DNA was diluted to working concentration of 10 ng/µl with deionized distilled water for polymerase chain reaction amplification (Foissac et al., 2001).

#### Polymerase chain reaction (PCR)

The diluted leaf extracted DNA was used as template for PCR amplifications to detect the presence of CMD as well as characterize the type of Cassava Mosaic Virus (CMV). Amplification was done with specific primers designed by Mills et al. (1992) (Table 2) to detect CMD (Fondong et al., 2000) (Table 3). To each tube of the PCR premix kit (1 unit of Hot start Tag, DNA polymerase 1x PCR Buffer containing 1.5 Mm $MgCl_2$ and 250 µl of each DNTP) was added 2 µl each of the 10 ng/ml DNA together with 1 µl each of both forward and reverse primers and 16 µl of distilled water to a total volume of 20 µl. Amplification of the reaction mixture was done using an AB1 2720 PCR thermocycler, with initial denaturation at a temperature of 94°C for 5 min. Denaturing step was achieved at 94°C for 30 s with annealing at 52°C for 60 s and extension at 72°C for 2 min. for 35 cycles, and final extension at 72°C for 5 min at 4°C.

#### Primers used

#### *Agarose gel electrophoresis*

PCR products were separated using 1.5% agarose gel prepared with TAE Buffer (242 g Tris base, 57.1 ml glacial acetic, 100 ml 0.5M EDTA). A total of 4 µl of ethidium bromide was added per 100 ml agarose gel solution for staining the DNA amplified fragments and visualization. Exactly 2 µl of 1× loading buffer was added to 10 µl of each DNA sample loaded into the prepared wells and ran with 1 × TAE buffer at 100 V under continuous current for 1.5 h. Size marker of 10 µl of 100 bp ladder was loaded in each first gel well and 10 µl of the positive samples containing 1000 bp ACMV and 1500 bp EACMV. After electrophoresis, the amplified bands were visualized under UV light transilluminator and photographed for data management.

## RESULTS AND DISCUSSION

Out of the 27 varieties tested, 24 clones were positive for ACMV, 3 were positive for EACMV-UG and one was asymptomatic for CMD (Table 3). Clones Korpoigibagie, Marie and Diamonyamawoi recorded mixed infection (Table 3). Findings of this study reveal that CMVs are very widespread in germplasm collected from farmers across Sierra Leone. The field assessments carried out at Months 1 and 3 showed that more than 80% of cultivars in the experimental plot had a score greater than one on the scale proposed by Legg et al. (2000); this is consistent with the high incidence values reported for some other countries in Africa (Ntawuruhunga et al., 2007; Thresh et al., 1997; Otim-Nape et al., 2000; Otim-Nape et al., 1993; Legg, 1999; Samura et al., 2016).

### Amplification

A total of 27 DNA samples of cassava were amplified for the EACMV-UG with the species-specific primer pair (UNIREP), but samples 1-15 did not generate any PCR product at 1500 bp. At 1000 bp, an amplicon showed

**Table 3.** Scores of molecular disease diagnostic for EACMV AND ACMV.

| S/N | Genotypes | EACMV-UG | ACMV | Designation |
|---|---|---|---|---|
| 1 | Warima | - | + | Local |
| 2 | Konko | - | + | Local |
| 3 | SLICASS 4 | - | + | Improved |
| 4 | Ya-Kanu | - | + | Local |
| 5 | Munafa | - | - | Local |
| 6 | Kendemeh | - | + | Local |
| 7 | Farannaka | - | + | Local |
| 8 | Mamoudukuma | - | + | Local |
| 9 | Improve | - | + | Local |
| 10 | Yangay | - | + | Local |
| 11 | Guineanca | - | + | Local |
| 12 | Samuyana | - | + | Local |
| 13 | Tapioca | - | + | Local |
| 14 | SLICASS 6 | - | + | Improved |
| 15 | Guawai | - | + | Local |
| 16 | Slicass11 | - | + | Improved |
| 17 | Kamawei | - | + | Local |
| 18 | Cocoa | - | + | Local |
| 19 | Milikit | - | + | Local |
| 20 | Korpoigibagie | + | + | Local |
| 21 | Marie | + | + | Local |
| 22 | Diamonyamawoi | + | + | Local |
| 23 | Tangayawa | - | + | Local |
| 24 | Nyon | - | + | Local |
| 25 | IDA | - | + | Local |
| 26 | Cooksoon | - | + | Local |
| 27 | Rubber | _ | + | Local |

+ = virus present; - = virus absent; EACMV-UG=1500 bp; ACMV = 1000 bp.

ACMV infection with the OjaRep species-specific primer (Figure 1). Using the same primers as shown in plate 1, samples 20 and 21 produced amplicon at same bp of EACMV-UG (Figure 2). After the electrophoresis of gel, the results showed the presence of both EACMV-UG (1500 bp) and ACMV (1000 bp) that cause CMD (Figures 1 and 2). Bands on the gel at 1500 bp representing EACMV-UG were very faint due to the high amplicon (1500 bp); the infected plant which served as positive control (+ve) shows a clear amplicon at the bp of the ACMV (1000 bp) but faint on the amplicon of the EACVM-UG (1500 bp).

The study indicates initial efforts to determine the occurrence of ACMV and EACMV-UG from across Sierra Leone. The high incidence of CMV in the germplasm obtained in this study agrees with previous studies on wide-spread CMD attack in cassava trials in Nigeria (Ogbe et al., 2003a; b, 2006). In this study, most positive samples also had high in-field symptomatic expression of the disease.

One variety (Munafa) appeared to have symptoms in the field, but no viruses were detected; this is possibly indicative of the absence of the virus in the young leaf or presence at a very low level. It is also possible that there were other diseases or viruses that were not tested for in the current study. The presence of two strains of viruses in the same plant agrees with the results of Fondong et al. (2000) and Harrison et al. (1997).

The current findings indicate that the dominant virus in Sierra Leone is ACMV but that EACMV is now also present. Farmers predominantly use stem cuttings for planting; the results show that even “improved” varieties are likely to be infected. Surveys conducted in Sierra Leone in 2016 confirmed the presence and rapid spread of CMD in the country (Samura et al., 2016). A correlation has been established between whitefly and cutting borne-infection in cassava genotypes found in other parts of Africa (Legg and Raya, 1998) and appears to hold true in Sierra Leone.

The results are similar with Monde et al. (2010), who reported the Ugandan strain (EACMV-UG) of the virus in DR Congo and Burkina-Faso, suggesting the migration of

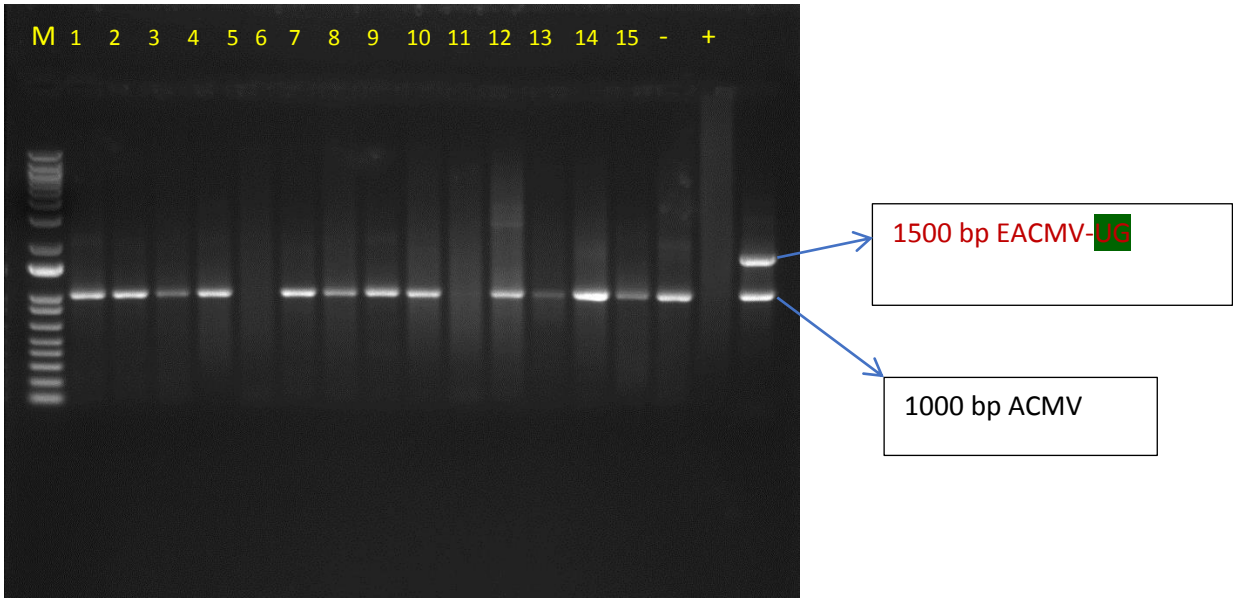


**Figure 1**. M = ladder, Lane 2 = Warima, Lane 3 = Konko, Lane 4 = Diamonyamawoi, Lane 5 = Slicass 4, Lane 6 = Ya-Kanu , Lane 7 = Munafa , Lane 8 = Kendemeh, Lane 9 = Farannaka, Lane 10 = Guawai, Lane 11 = Mamoudukuma, Lane 12 = Silcass 11, Lane 13 = Kamawei, Lane 14 = Tangayawa, Lane 15 = Milikit, Lane 16 = negative control, Lane 17 = positive control.

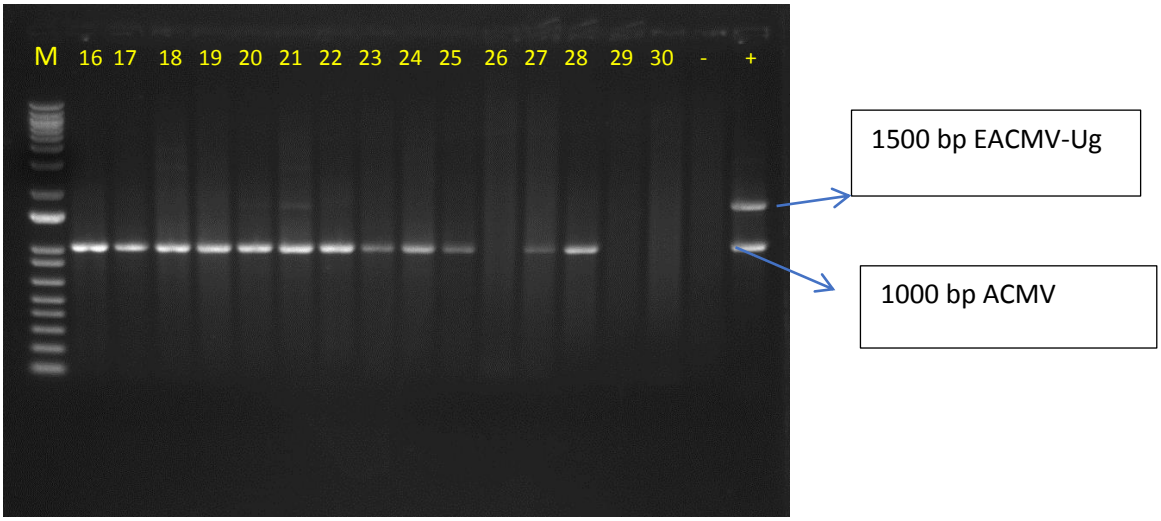


**Figure 2.** M = ladder, Lane 16 = Slicass11, Lane 17 = Kamawei, Lane18 = Milikit, Lane 19 = Korpoigibagie, Lane 20 = Cocoa, Lane 21 = Marie, Lane 22 = Diamonyamawoi, Lane 23 = Tangayawa, Lane 24 = Nyon, Lane 25 = IDA, Lane 26 = Cooksoon, Lane 27 = Rubber, Lane 17 = negative control, Lane 18 = positive control.

the virus from East to West Africa (Tiendrebeogo et al., 2009). The route of the disease might further confirm the movement of the pandemic from East to West Africa either by long-distance distribution through infected stem cuttings or by whitefly *Bemisia tabaci*. The morphological identification of CMD (Figure 3) showed that most genotypes had a consistently increasing disease incidence between 3 and 6 months after planting (MAP), except genotypes Guineanca, SLICASS 11 and Mamouduk, which recorded equal incidences at 3 and 6 MAP.

This study registered the existence of the endemic related recombinant virus, EACMV-UG, in certain varieties in the country and confirmed the similarity in an earlier report of the virus in ROC (Neuenschwander et al., 2002). However, variations in whitefly *Bemisia tabaci*

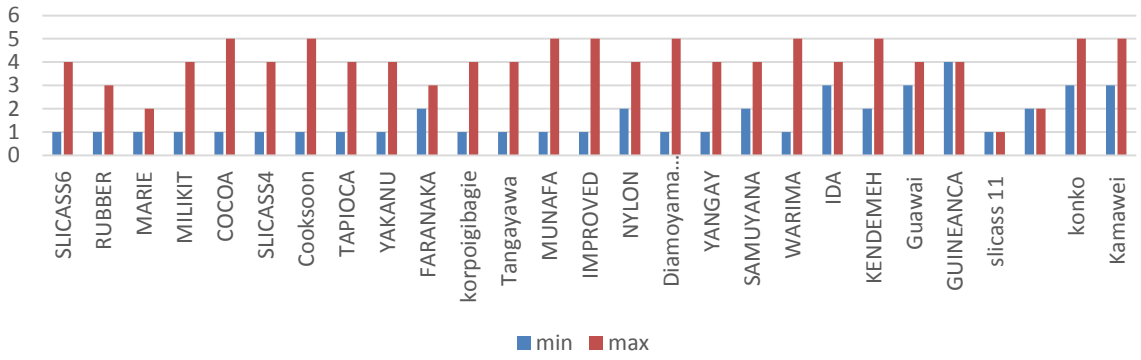


**Figure 3.** Mophological Identification of CMD.

occurrence (Fishpool et al*.,* 1995) and their transmission resulted in difficulty in identifying the first visible symptom expression within three to five weeks after planting (Fargette et al*.*, 1993). A frequency study to know the population dynamics would be needed to understand the relationship between the CMD spread and vector population in Sierra Leone. Results show symptom severity differences among regions, but not as severe as the general disease situation in other countries (Sseruwagi et al., 2004b). This could probably be attributable to high mixed virus infection frequencies through synergistic interactions (Harrison et al*.,* 1997; Fondong et al., 2000; Pita et al., 2001; Ntawuruhunga et al., 2007). Even though ACMV is seemingly the most frequently occurring virus in Sierra Leone, some of the sampled plants had mixed infection of ACMV and EACMV-UG. More research needs to be done across the entire country to know if the proportion has increased as there was a positive result found in some of the varieties studied. Large proportions of the landraces sampled throughout the study were highly diseased with CMD. These results were consistent with an earlier report on the pattern of CMD infection of cassava landraces (Ntawuruhunga et al., 2007). Improved cassava varieties (that is, Slicass 4, Slicass 6 and Slicass 11) studied here had positive infection of CMD, suggestive of a possible breakdown of resistance in some improved varieties and the need for backcrossing to recover the resistant gene of the donor parent in the progeny. A CMD management strategy needs to be implemented in Sierra Leone based on the multiplication and dissemination of CMD-free planting materials with strict phytosanitary programs to minimize the spread of the mosaic virus. Similar measures were undertaken in East Africa and in Republic of Congo (Ntawuruhunga et al., 2007; Legg, 1999; Otim-Nape et al., 2000).

## Conclusion

Samples were collected from the four main agro-ecologies in Sierra Leone. Samples included 24 local varieties and three improved varieties. Only one of 27 varieties tested negative for CMV; unfortunately this variety (Mafuna) showed symptoms identical to CMD in the field. Plants with CMD were found in all regions sampled. The predominance of using infected cuttings from the varieties collected from the three regions in this study could indicate that CMD pandemic has covered the whole country. The three improved cassava varieties (which are the most commonly encountered improved varieties) tested positive for CMV; this suggests the need to engage in some backcrossing to recover the resistant gene of the donor parent in the progeny. Because of its wide-spread occurrence, CMD is a limiting factor on cassava yield in Sierra Leone. There is urgent need for plant breeders to develop resistant strains, and farmers must be educated in appropriate techniques if Cassava Mosaic Disease mitigations are to be achieved in Sierra Leone.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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