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Status and Diversity of the Cassava Mosaic Disease Causal Agents in Sierra Leone

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Abstract Cassava is the most important root and tuber crop in Sierra Leone. Its low yield can be attributed to several production-limiting factors including cassava mosaic disease (CMD). This study examined in a much wider scope the diversity, prevalence, distribution, incidence and level of severity of the CMD within the cassava farming communities across major agro ecologies in Sierra Leone. A survey was conducted October, 2010. Field assessment was also conducted on farms evaluated. Data was collected on the spot and complimented with group discussions and interviews. Field coordinates were determined using a global positioning system (GPS) recorder. This study showed a countrywide prevalence of 85.2% out of 156 sites visited using GPS mapping. The rain forest ecology had the highest prevalence of 97.2% while the coastal plain had the lowest disease prevalence. Incidence of CMD per district was generally high. Tonkolili district recorded the highest incidence of 99.2% followed by Kailahum and Pujehun. Bonthe district had the lowest severity score, while pujehun district had the highest severity score. Difference in CMD infection was also observed in terms of agro-ecology. Test using polymerase chain reaction (PCR) detected African cassava mosaic virus (ACMV) and also for the first time the East African cassava mosaic virus (EACMV) in two locations in the Moyamba district, southern Sierra Leone. The result from this study indicates the need for an increased adoption of CMD resistant cassava genotypes that are high yielding, has good cooking quality and with the ability to replace the local choice variety without significantly altering the cultural and aesthetic quality of the generally accepted local cultivar.

Keywords Incidence, Severity, Diversity, Cassava mosaic geminivirus

1. Introduction

Cassava (*Manihot esculenta* Crantz) is the second most important food crop after rice in Sierra Leone; it is also the most important root and tuber crop [21]. Cassava is also grown all over the country which has shown remarkable success in its processing at both domestic and commercial scales. Cassava was introduced to Africa by the Portuguese during the late 16th century [18]. It was readily adaptable to the different environmental conditions and well suited for integration into the farming systems and socioeconomic conditions of Africa. In Sierra Leone, cassava is processed into some common products: *gari*, *lafun*, starch and boiled cassava with beans. Cassava-based products such as raw tubers, *gari* and cassava bread (very thin, small, flat, round pieces) are traded mainly in Sierra Leon [14]. Cassava leaves provide source of income for women. The leaves are used to prepare a very popular national cassava leaf sauce [20].

Average yield of cassava in Sierra Leone is low and is estimated at 7.18 t/ha according to FAO 2012. [8]. The low yields of cassava in Sierra Leone can be attributed to several production-limiting factors. These include, among others diseases, pests and weed infestation, as well as edaphic, agronomic and socio economic factors [1]. Diseases have been shown to play a prominent role in limiting cassava productivity in Sierra Leone. Some of those of economic importance include the cassava mosaic disease (CMD), cassava bacterial blight (CBB), and recently cassava brown streak virus [5].

The CMD is endemic to Africa, caused by at least 7 different species of whitefly (*Bemisia tabaci*) transmitted geminiviruses, commonly referred as cassava mosaic geminiviruses (CMGVs) [16]. They are likely descendants of geminiviruses adapted to infect indigenous uncultivated African plant species [9]. Therefore the adaptation of CMGV's to cassava could have only commenced, either after cassava was introduced to West Africa in the Gulf of

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Guinea during the 16th century, or after it was introduced to East Africa and the SWIO islands in the 18th century [9], [13].

Since the initial characterization in the early 1980s of the "first" CMG species, African cassava mosaic virus (ACMV) [3], it has subsequently been discovered that African CMG's in fact consist of at least seven distinct begomovirus species including South African cassava mosaic virus (SACMV), East African cassava mosaic virus (EACMV), East African cassava mosaic Cameroon virus (EACMCV), East African cassava mosaic Zanzibar virus (EACMZV), East African cassava mosaic Malawi virus (EACMMV), and East African cassava mosaic Kenya virus (EACMKV) [4], [15]. Recently, two species have also been newly described: African cassava mosaic Burkina Faso virus (ACMBFV) and Cassava mosaic Madagascar virus [23]

Several species and strains of cassava mosaic geminivirus have been described [10]. Limited information on cassava mosaic viruses have been provided from Sierra Leone in terms their distribution and none on their effects on growth or yield. These are serious deficiencies and thus emphasize the inadequate attention given to the cassava mosaic viruses in Sierra Leone which affect the most important root and tuber crop. The traditional belief that mosaic infected cassava genotypes act an indicator for the identification of poundable cassava genotypes with high dry matter content has encouraged the use of locally infected genotypes. Further there is a popular demand for mild mosaic infected cassava leaves among women for preparing a popular vegetable sauce using cassava leaves. It is believed that the infected leaves due to the low chlorophyll content consumes less palm oil, an expensive but crucial element in the preparation of the cassava leaf sauce compared to improved resistant varieties which consumes more. Women play a vital role in the selection of planting materials and would influence varieties of their choice which in most cases would include infected local varieties for domestic consumption.

Results of preliminary surveys carried out by [22] indicated that ACMV is prevalent throughout the western areas of Sierra Leone with an estimated 100% incidence in smallholder farms. On the spot check on farmers' field in other part of the country such as Bo, Kemena, Pujehun, and Njala indicate similar trends which warrant a nationwide survey on the prevalence and distribution of the mosaic virus as well as the diversity of the CMGVs.

The large molecular diversity with viruses from east Africa points to the region as the centre of diversification [10]. In addition and more significantly recombination evident in a number of virus genomes is a driving force of geminivirus evolution. Virus diversity and frequent recombination events found in virus genomes provide evidence for continuous evolutionary processes and influence the development of epidemics and the emergence of "new" viruses. The knowledge of virus diversity, the geographic distribution of virus types and the structure of virus populations is a significant prerequisite to deploy cassava with virus resistance characters.

This study examined in a much wider scope the different CMGVs and their distribution within the cassava farming community in Sierra Leone. Elucidation of the current prevalence and distribution of species/strains of the CMGVs will provide new opportunities for developing effective interventions. These include developing cassava genotypes with appropriate source of resistance as well as farmer' desired traits for the prevailing environment and subsequently increase yields and productivity of cassava. The study was therefore undertaken with the following objectives in mind:

1. To identify diversity among cassava mosaic geminivirus group within Sierra Leone
2. To determine prevalence, incidence, severity and distribution of CMV in Sierra Leone

2. Materials and Method

2.1. Survey Routes

The survey was conducted between October and November 2010. The survey routes were determines using the road maps of Sierra Leone and such routes includes highways, secondary roads and feeder roads. The route was selected to target major cassava growing area within the geo political districts as well as the major agro ecologies in Sierra Lone.

2.2. Field Assessment

Sample sites were selected along the route based on diversity of the villages as well as farms in order to capture diversity of the cassava mosaic virus. Approximate age of the crop/plants (in months), variety, origin of stem cuttings, and duration of the cultivar was investigated. Group discussion and interviews was conducted to compliment field data. This ranged between 2-3 km in smaller chiefdoms and 3-5 km in bigger chiefdoms. A total of 156 fields were visited. In each field, the coordinates were recorded using the Global positioning system (GPS) GARMIN e Trex Legend 1200 E 151st Street, Olathe, Kansaa 66062 U.S.A).

2.3. Disease Assessment

On the spot assessment was conducted in all fields in the western Area and 12 districts and 5 agro- ecologies which include the rain forest, savannah lowland, savannah highland, coastal plains and the peninsular mountain. Prevalence was calculated as the number of site infected over the total number of sites visited expressed in percentages. Leaf sample was collected from the dominant variety grown and from other plants showing distinct diversity. Incidence and severity of the cassava mosaic virus was assessed using the five point scale adopted by [12] as follows

1 = Symptom-less plants

2 = Mild chlorotic patterns affecting most leaves; mild distortions at the bases of most leaves and remaining part of the leaves are normal

3 = Pronounced chlorosis on most leaves, narrowing

and distortion of the lower one-third of the leaflets.

4 = Severe chlorosis and distortion of two-thirds of most leaves and general reduction of leaf size and some stunting.

5 = Most severe symptoms (severe chlorosis, leaf distortion, twisting, misshapen leaves, severe reduction of most leaves and severe plant stunting)

Percent incidence was calculated by expressing in percent the total number of infected plant over the total number of plants sampled.

5 Leaf sample was collected from each field for diverse symptom types; severe, moderate, mild, no symptoms).

The field with an overall impression of symptom severity score 2 was regarded as mild, score 3 as moderately severe and 4 or 5 as severe. Infection type was determined as,

A) Cutting-borne: Where all leaves of the infected plants show uniform symptoms.

B) Whitefly-borne: Where symptoms may not be apparent on old leaves (bottom portion of the plant), but clear symptoms on newly produced leaves (top portion of the plant).

C) Super infection: Where different symptoms in the same plant. Old leaves (bottom portion) show mild or moderate symptoms; and newly emerging leaves (top portion) show severe symptoms (symptoms very different from the lower portion).

Adult whitefly was counted from the lower side of the leaf on the five topmost fully-formed apical leaves of the tallest shoot on ten randomly selected plants diagonal to the farm's orientation.

Leaf sample was collected based on the diversity of symptom expression as described above. In transit leaf sample was stored in 4℃ refrigerator prior to analysis at the Virology and Molecular and Diagnostic Unit at the International Institute of Tropical Agriculture (IITA) for analysis.

2.4. Laboratory Analysis of Samples

2.4.1. DNA Extraction

Total DNA was extracted from eighty cassava leaf samples according to the procedures of [6]. Extracted DNA from leaf sample was resuspended in 200µl TE (Tris- HCL 50mM, EDTA 10mM) and p H 8.0 and stored at -20℃.

2.4.2. Testing of DNA Samples

Leaf samples were tested by polymerase chain reaction (PCR). DNA from leaf samples was diluted to obtain 2 ng/µl. The specific primers for the detection of ACMV, EACMV, ACMV + ECMV and EACMV – UG was used as represented in Table 1.The reaction mixture per tube contained 2.5 µl of the Green buffer (10 x concentration), 0.75 µl MgCl2; 0.25 µl of dNTP, 0.25 µl each of forward and reverse primers, 0.06 µl of taq DNA polymerase (Promega, USA) 6.44 µl sterilized distilled water, and 2.2 µl of healthy cassava were used as negative control. The healthy negative control clone was obtained from virus tested plant. EACMV DNA (obtained from the virology and molecular diagnostic unit IITA Ibadan) was used as a positive control for the detection of the virus.

The reaction cycles using a PTC DNA Engine system (model PTC 200, MJ Research Inc., 149, Grove Street, Watertown, Massachusetts USA) were as reported by [24]. The first cycle consisted of 1 min at 94℃, 2 min at 52℃ and 3 min at 72℃. This was followed by 35 cycles of 1 min 94℃, 1 min at 52℃, and 1.33min at 75℃. The final cycle consisted of 5 min 72℃. The PCR products were separated by electrophoresis in a 1% agarose gel, which contains 1.5ul of ethidium bromide (10mg/ml) in Tris – acetate –EDTA (TAE)buffer (0.04 M Tris acetate p H 8.0 + 0.01 M EDTA, pH 8.0) at 100 volts for about 1.5 hr. The DNA bands were observed under UV light and positive and negative reactions were recorded.

Table 1. Nucleotide sequence of DNA primers used in polymerase chain reaction for the detection of African cassava mosaic virus (ACMV), East African cassava mosaic virus(EACMV), Uganda variant of EACMV (EACMV-Ug2) [a]

Virus	Name of Primer	Sequence (5' to 3')	Target in DNA[b]
ACMV	ACMV –ALI/F	GCG GAA TCC CTA ACA TTA TC	AC1
	ACMV-ARO/R	GCT CGT ATG TAT CCT CTA AGG CCT G	AV2
	JSP001	ATG TCG AAG CGA CCA GGA GAT	CP
	JSP002	TGT TTA TTA ATT GCC AAT ACT	CP
EACMV	JSP001	ATG TCG AAG CGA CCA GGA GAT	CP
	JSP 003	CCT TTA TTA ATT TGT CAC TGC	CP
EACMV –Ug2	UV-AL1/F	TGT CTT CTG GGA CTT GTG TG	AC1
	ACMV-CP/R3	TGC CTC CTG ATG ATT ATA TGT C	CP

[a]Sources of primer sequence: [7], [2]; [24]; [19] . The primers were used in pairs as listed, the first being forward primer and the second being the reverse primer
[b]AV = DNA-A virus sense (AV2:gene function not yet known), AC = DNA –A complementary sense (AC1:replication initiation protein gene, AC3: replication enhance protein gene), CP = coat protein gene.

3. Result

3.1. Prevalence of the Cassava Mosaic Disease in Sierra Leone

Country wide the prevalence of cassava mosaic disease was 85.2% out of 156 sites visited. The Rain forest ecology comprising Kono, Kenema and Kailahum had the highest prevalence of 97.2% while the coastal plain had the lowest disease prevalence of 45.8%. On district bases, disease prevalence was high. Moyamba, Kono, Kailahun and Tonkolili districts had 100% prevalence of the disease while Bonthe district has considerably the lowest prevalence of 16.6%. (Table 2).

Table 2. Prevalence of the Cassava Mosaic Disease in Sierra Leone

Agro ecology	Prevalence (%)	Districts	Prevalence
Savannah Lowland	93.0	Kambia	91.6
		Bombali	83.3
		Moyamba	100
		Tonkolili	100
		Pujehun	91.6
		Bo	91.6
Savannah High land	91.6	Koinadugu	91.6
Coastal Plain	45.8	Bonthe	16.6
		Port Loko	75
Rain Forest	97.2	Kono	100
		Kenema	91.6
		Kailahun	100
Peninsular Mountain	75.5	Western Area	75.5

3.2. Incidence of CMD per District

Incidence of CMD per district was generally high. Tonkolili, Moyamba, Kono, and Kailahum district recorded the highest incidence of 100.2% followed by Bo, Kambia Kidence enema, Koinadugu and Pujehun districts with 91.6% incidence, respectively. Bonthe district had lowest disease incidence of 16.67% followed by Port Loko and Western Area with 75% respectively (Table 3).

Table 3. Incidence and severity of Cassava Mosaic Disease (CMD) per District

District	Incidence	Severity
Kambia	91.6	2.68
Bo	91.6	2.78
Bombali	83.3	2.87
Bonthe	16.6	1.26
Kailahun	100	2.62
Port Loko	75	2.2
Kenema	91.6	2.73
Koinadugu	91.6	2.48
Kono	100	2.67
Moyamba	100	2.5
Pujehun	91.6	3.74
Tonkolili	100	2.74
Western Area	75	2.93
CV (%)	35.0	31.6
SE(±)	29.78	0.83

3.3. Severity of Cassava Mosaic per District

Significant difference was observed within district in their response to CMD ($P<0.05$). Generally severity of CMD was mild based on the criteria adopted for severity ranking. Bonthe district had the lowest severity score of 1.26 which was considered to be resistant. Pujehun district had the highest severity score of 3.74 and was classified to be severe. Severity score of other districts ranged between 2.4 to 2.9 which were considered to be mild (Table 3).

3.4. The Distribution of Farms Showing Incidence and Severity of CMD in Sierra Leone

CMD was widely distributed country wide with 112 (73.2%) of farms having an incidence of ranging between 81 to 100%. 26 (16.9%) of the farms surveyed had disease incidence of 20% and less (Fig 1).

Severity of cassava mosaic disease was generally mild.

16(10.45%) farms had high severity rating of 4.0 to 5.0 which was mostly observed in Pujehum, western Area and some parts of Kambia and Bombali. 28 (18.1%) of the farms assessed had low severity rating between 1 to 2 and was observed mostly in Bonthe, some area in Bo, Bombali, Port Loko, Western Area, Kenema, Koinadugu and Kambia districts. 62 (40.5%) of the farms surveyed had severity scores between 2 to 3 (Fig 2).

3.5. CMD across Agro Climatic Ecologies in Sierra Leone

Incidence and Severity of CMD

The Savannah lowland had the highest disease incidence of 89% followed by the Rain forest with 87.5%. The lowest disease incidence of 36.7% was observed in the coastal plain. In terms of disease severity, the rain forest zone had the highest severity score of 2.67 followed by the savannah lowland with 2.5. The coastal plain had the lowest severity score of 1.64. Based on the criteria adopted for resistance and susceptibility CMD severity was regarded as mild in all agro ecologies Table 4.

Table 4. Mean Incidence and severity of CMD across Agro - ecological zones in Sierra Leone

Agro climatic ecologies	Incidence of Cassava mosaic disease (%)	Severity of Cassava mosaic disease
Rain forest	87.5	2.67
Savannah Lowland	89.0	2.5
Savannah Highland	80.8	2.4
Coastal Plain	36.7	1.64
Peninsular mountain	73.0	2.9

Percent number of farms with improved and infected cassava genotypes

Generally, the number of farms with local varieties was higher (85%) than those with improved varieties (15%) during the survey from a total of 153 farms assessed. Bonthe district had the highest number of farms with improved varieties (83.33%) from a total number of 12 farms surveyed with 16.7% of the farms with local varieties. The Moyamba, Tonkolili, Kono, kailahun districts had no farm with improved varieties. All farms visited were planted with infected local varieties. This trend was observed in Bo, Kambia, Pujehum and Koinadugu with 8.3% of the farms with improved varieties and 91% of farms with local infected varieties (Fig. 3).

3.6. Diversity of Cassava Mosaic Geminiviruses among Local Cassava Varieties in Farmer's Field in Sierra Leone

Among the 80 leaf samples collected from symptom bearing plants, 70% tested negative to ACMV and EACMV. 28% of the samples tested positive for ACMV alone while 2% tested positive for EACMV.

The most common cassava mosaic geminivirus found within cassava field from leaf samples was the African cassava mosaic virus (ACMV) which is dominant in West Africa. Out of eighty samples tested 20 were positive for ACMV infection alone while 2 samples at Jawoma and Njala tested positive for East African Cassava Mosaic Virus (EACMV) and ACMV. Symptom expression in the field did not always match PCR test. Severe symptom was expressed with EACMV infection and had a severity score of 4 while ACMV infection alone was comparatively lower with a score of 3.

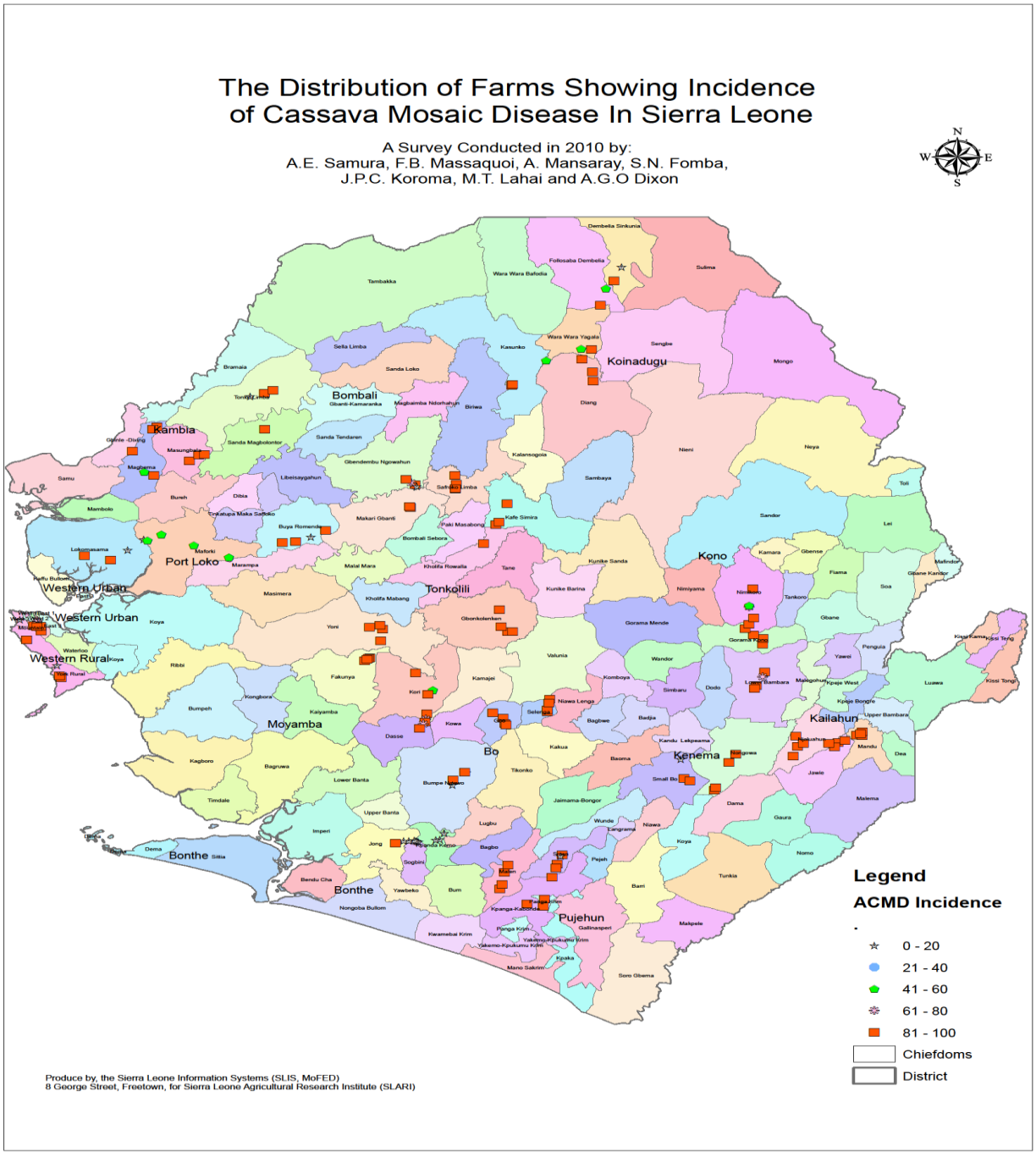

Figure 1. Distribution of Farms Showing Incidence of cassava Mosaic Disease in Sierra Leone

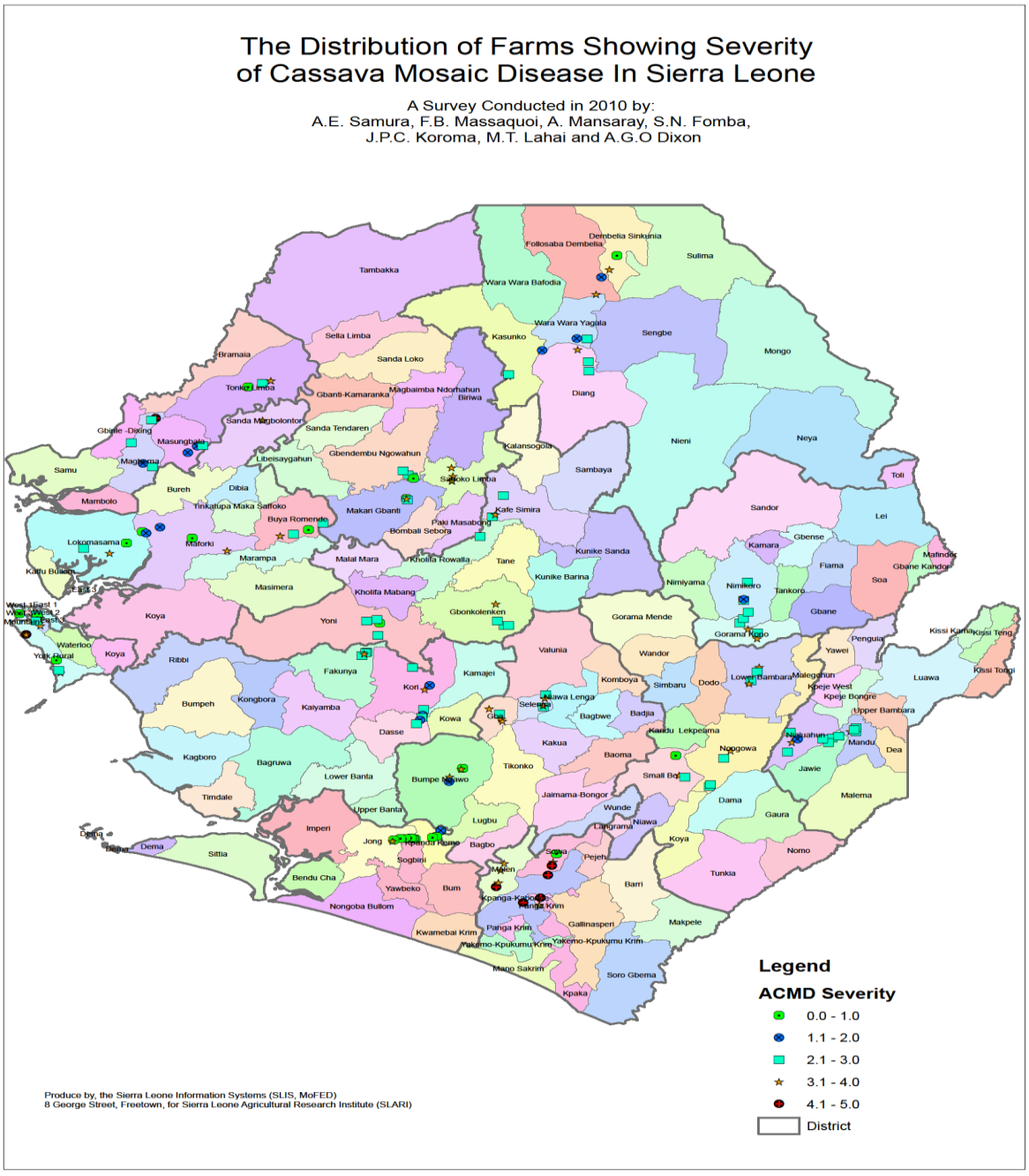

Figure 2. Distribution of Farms Showing Severity of cassava Mosaic Disease in Sierra Leone

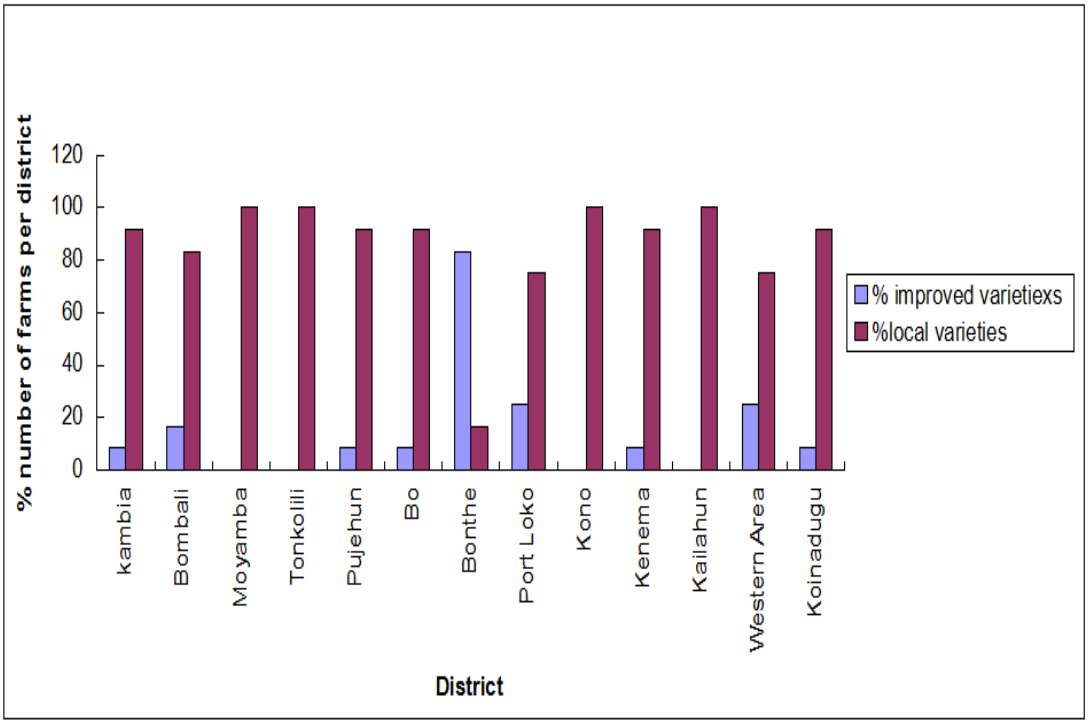

Figure 3. Percent number of farms with improved and infected local varieties per district in 2010

Table 5. Incidence, Severity and Diversity of the Cassava Mosaic virus using polymerase chain reaction (PCR)

Village	Repeat PCR			Incidence	severity
	ACMV	EACMV	EACMCV		
Fakunya	+++	-	not	100	4
Petema	+++	-	nt	60	3
Njala	+++	-	nt	100	4
Bailargo	+++	-	nt	100	4
Makeni	+++	-	nt	80	3
Kambia 2	+++	-	nt	100	4
Malikaya	+++	-	nt	100	4
Jawoma	+++	-	nt	100	4
Njala	+++	-	nt	80	3
Jawoma	+	+++	-	100	4
Segbwema	+++	-	nt	80	3
Segbwema	+++	-	nt	100	4
Rogbereh bridge	+	-	nt	70	3
Mabama	++	-	nt	40	2
Fakunya	+++	-	nt	100	4
Dogonklieh	+++	-	nt	100	3
Makeni	+++	-	nt	100	4
Beudu Junction	+++	-	nt	100	4
Mojoebobor	++	-	nt	100	3
Beudu Junction	++	-	nt	70	3
Makeni	+++	-	nt	100	4
Njala	+++	+++		100	4

All plants from which samples were collected were associated with Cutting-borne infection where all leaves of the infected plants show uniform symptoms. This result shows that CMD in Sierra Leone is mostly spread through infected cassava cuttings (Fig.4).

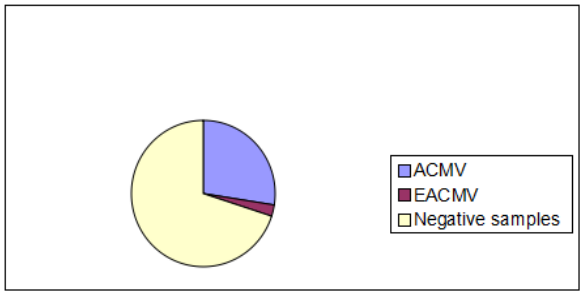

Figure 4. Proportion of samples positive to cassava mosaic genimiviruses

4. Discussion and Conclusions

The high prevalence of CMD in Sierra Leone can be attributed to the widespread use of local varieties susceptible to the disease. In contrast however the Bonthe district which recorded a disease prevalence of 16.6% can be associated with the cultivation of a released variety SLicass 4 commonly referred to as blue boat. At the time of this survey, Puejun district had the highest disease severity score of of 3.74 and a prevalence rate of 91%. Since most of the infection is caused by the use of infected cutting breeding effort for control of the CMD should be geared towards

replacing infected local varieties with farmer desired genotypes that are resistant to the disease. The adoption of Slicass 4 (Blue boat) in Bonthe is closely linked with high level of gari production. It can be concluded that the adoption of disease resistant varieties must be complimented by improved processing facilities. Efforts have been made to disseminate improved high yielding cassava genotypes which may have being captured during the survey as indicated in the incidence and severity map (Fig 1 & 2). This effort has to be compliment with functional processing facilities for meaningful impact to be in the control of the mosaic virus. Additionally the application tissue culture technique through in vitro multiplication of virus free plants for distribution to farmers may help reduce the prevalence rate of the disease as well as maintaining the genetic diversity of indigenous local varieties. It was widely believed that ACMV was the only species associated with cassava in Sierra Leone. This study provides information on the first report of the EACMV in Sierra Leone. In this survey only two samples tested positive for EACMV. Both samples were collected from the Moyamba district at Jawoma and Njala and were closest to the Njala Agricultural Research Center (NARC) which hosts the highest cassava germplasm collection in the country. This result is consistent with results observed in Nigeria where 0.05% of a total of 290 [17] and 0.3% out of a total of 1106 samples tested positive for EACMV. Plants with symptoms that tested negative to either ACMV and /or EACMV may be as a result of mistaken symptoms of cassava green mite which has a common symptom with CMD. However further studies are necessary to determine the virus diversity by sequencing for through knowledge on viruses in the country.

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