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VIRUSES ASSOCIATED WITH CASSAVA MOSAIC DISEASE AND THEIR ALTERNATIVE HOSTS ALONG NIGERIA-CAMEROON BORDER

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ABSTRACT

Cassava mosaic disease (CMD) threatens cassava production across the African continent. Because CMD is widespread in sub-Saharan Africa (SSA), it is particularly important to monitor border regions to prevent the introduction of other cassava (*Manihot esculenta* Crantz) viruses and strains into regions otherwise considered still free of the viruses. The objective of this study was to establish the occurrence of viruses associated with CMD and their alternate host plants along the Nigeria-Cameroon border. One hundred leaf samples from cassava plants and weed species were collected across 21 locations, along the border regions of Cross River State; and assessed for CMD incidence and severity. Nucleic acid extracts were obtained and used to test for African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV), using polymerase chain reactions. Plants from ten, seven and four locations had mild, moderate and severe CMD symptoms, respectively. Of the 100 samples collected, 68 were symptomatic and 32 were asymptomatic for CMD; while five weed species were symptomatic. ACMV alone was detected in six symptomatic and three asymptomatic cassava leaves. EACMV was singly detected in one symptomatic and two asymptomatic cassava leaves; while 13 cassava leaves from ten locations had mixed ACMV/EACMV infections. One asymptomatic cassava leaf from Ikang tested positive for ACMV and EACMV. EACMV alone was detected on a symptomatic weed species, *Sclerocarpus africanus*. The occurrence of viruses causing CMD in Nigeria, including the border regions, has now been further documented, and *S. africanus* is now verified as a host of EACMV. There is a need for further studies on other plants that may be putative reservoirs for cassava mosaic viruses, towards the development of integrated management strategies.

Key Words: *Manihot esculenta*, *Sclerocarpus africanus,* weeds

RÉSUMÉ

La maladie de la mosaïque du manioc (CMD) menace la production de manioc sur tout le continent africain. Étant donné que la CMD est répandue en Afrique subsaharienne (ASS), il est particulièrement important de surveiller les régions frontalières pour empêcher l'introduction d'autres virus et souches du manioc (*Manihot esculenta Crantz*) dans des régions autrement considérées comme encore exemptes de virus. L'objectif de cette étude était d'établir la présence de virus associés à la CMD et de leurs plantes hôtes alternatives le long de la frontière Nigeria-Cameroun. Cent échantillons de feuilles de plantes de manioc et d'espèces de mauvaises herbes ont été prélevés dans 21 endroits, le long des régions frontalières de l'État de Cross River ; et évalués pour l'incidence et la gravité de la CMD. Des extraits d'acide nucléique ont été obtenus et utilisés pour tester le virus de la mosaïque du manioc africain (ACMV) et le virus de la mosaïque du manioc d'Afrique de l'Est (EACMV), en utilisant des réactions en chaîne par polymérase. Les plantes de dix, sept et quatre emplacements présentaient respectivement des symptômes de CMD légers, modérés et graves. Sur les 100 échantillons prélevés, 68 étaient symptomatiques et 32 étaient asymptomatiques pour la CMD ; tandis que cinq espèces de mauvaises herbes étaient symptomatiques. L'ACMV seul a été détecté dans six feuilles de manioc symptomatiques et trois asymptomatiques. L'EACMV a été détecté individuellement dans une feuille de manioc symptomatique et deux asymptomatiques ; tandis que 13 feuilles de manioc provenant de dix sites présentaient des infections mixtes ACMV/EACMV. Une feuille de manioc asymptomatique d'Ikang a été testée positive pour l'ACMV et l'EACMV. EACMV seul a été détecté sur une espèce adventice symptomatique, *Sclerocarpus africanus*. La présence de virus causant la CMD au Nigeria, y compris dans les régions frontalières, a maintenant été davantage documentée, et *S. africanus* est maintenant vérifié en tant qu'hôte de l'EACMV. Il est nécessaire de poursuivre les études sur d'autres plantes qui pourraient être des réservoirs putatifs pour les virus de la mosaïque du manioc, en vue de développer des stratégies de gestion intégrée.

Mots Clés: *Manihot esculenta*, *Sclerocarpus africanus*, mauvaises herbes

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a crop of global significance being a principal source of energy and proteins for humans and livestock; and as a raw material for a spectrum of industries. In sub-Saharan Africa (SSA), it is consumed in the forms of roots and leaves. The resilience of cassava to adverse climatic conditions enables the crop to grow successfully under a wide range of agroecological zones, where other principal food crops cannot thrive. This makes cassava a food security crop for poor farmers who cultivate under marginal environments, particularly in SSA (Umunakwe *et al.,* 2015). However, cassava production in Africa has been limited by a variety of diseases, especially cassava mosaic disease (CMD) (Legg *et al.,* 2015).

Cassava mosaic geminiviruses (CMGs), belonging to the genus *Begomovirus,* are the actual causative agents of CMDs (Ndunguru *et al.,* 2005), a common disease that limits cassava production in countries like Nigeria (Nweke *et al.*, 1994). The disease is prevalent in most cassava farms, causing substantial yield reductions, although total losses are difficult to estimate, particularly along national borders. Yield losses in cultivars from different countries range from 20 to 95% (Thresh *et al.,* 1994); while some CMG strains produce more severe symptoms than others, with greater effects on cassava growth and yield (Owor *et al.,* 2002). Studies in Cameroon and Uganda revealed that dual infection with two different CMGs caused more severe symptoms than either virus alone (Fondong *et al.,* 2000a; Pita *et al.,* 2001).

One of the key gaps in knowledge on the epidemiology of CMD in Nigeria is the potential role of non-cassava plant species as alternate or reservoir hosts in the perpetuation of CMGs. Initially, the natural hosts of CMGs were largely within the family Euphorbiaceae, although it can be experimentally transmitted to members of the family *Solanaceae* (Bock and Woods, 1983). In addition, CMGs are not known in the centre of origin of cassava, from where they were introduced into Africa. Thus, the virus may have been present in some indigenous hosts and became pathogenic on cassava upon its introduction to Africa. This is further supported by the identification of some indigenous weeds as CMG hosts (Alabi *et al.,* 2008a). Therefore, understanding the alternate hosts of CMGs in any cassava growing environment will help in developing appropriate integrated management strategies for this disease. Although East African cassava mosaic virus (EACMV) has been reported to occur in natural hosts other than cassava in Nigeria (Alabi *et al.,* 2007), the most dominant virus is the African cassava mosaic virus (ACMV), as observed in previous diagnostic surveys (Ogbe, 2001; 2003).

The exchange of planting materials across country borders generally facilitates the spread of otherwise quarantined pathogens from country to country (Kolade *et al.,* 2022). New virus entry into Nigeria, especially across the borders, may arise from neighbouring countries due to seed traded by the farmers living along those areas. There is, therefore, need for constant surveillance along country borders to ensure that the targeted country retains its low epidemic index, especially as the risk of spread for other cassava diseases exists. The objective of this study was to establish the occurrence of viruses associated with CMD and their alternate host plants along the Nigerian border with other countries.

METHODOLOGY

Study sites. This study was conducted along the Nigeria-Cameroon border of Cross River State, Nigeria. This is an area located within latitude 4º 79' to 6º 61' and longitude 8º 49' to 9º 44', at an altitude ranging from 19 to 271 meters above sea level. It comprises primarily of rain forest and guinea savannah agro-ecologies, receiving mean annual rainfall of 2,170 to 3,300 mm and temperatures of 24 to 29 °C. The area was divided into 21 sampling sites (Table 1).

Sampling procedure. Cassava leaves were sampled using predetermined routes based on the road map of Nigeria (Fig. 1). The total Nigeran-Cameroon border is estimated to be 1,975 Km long, although the route selected covered only the area within the Cross River State axis. Figure 1 displays the geographical location of the sites; while Table 1 shows the details for specific information recorded from the locations.

A maximum of six cassava leaf samples, consisting of one asymptomatic and five symptomatic (mild and severe) leaves from the fully expanded top five of the tallest shoots, were collected across the sites, and preserved in separate microtubes. The microtubes were labelled and stored in sample cooler boxes containing ice blocks. Cassava stem cuttings were also collected from plants where leaves were sampled.

Collection of samples from the field was performed by trekking using an 'X' design as described by Sseruwagi *et al.* (2004). Leaf samples were additionally collected from symptomatic and asymptomatic weeds with fully developed floral parts; or suspected to be infected with cassava mosaic viruses. All the samples were transferred to the molecular breeding and diagnostic laboratory of the National Root Crops Research Institute, Umudike, Abia State. The samples were stored at -20 °C until analysis, and the stem cuttings were planted in pots so that infected leaf samples could be retrieved from them for further investigations.

Disease incidence and severity. In each field, a maximum number of 30 cassava plants

TABLE 1. Locations sampled for the occurrence of cassava viruses along the Nigeria-Cameroon border

Locations	LGA[a]	Longitude	Latitude	Altitude (m)	NPS[b]	NCLO[c]	NOW[d]
Ikang 1	Bakassi	8.52695	4.79442	20.4	30	8	2
Ikang 2	Bakassi	8.52484	4.7945	18.9	30	4	0
Ikang 3	Bakassi	8.5109	4.80662	33.3	30	6	0
Edik Idim Ikot Eyi	Bakassi	8.48981	4.81931	51.9	30	5	0
Edik Idim Ikot Nsid	Bakassi	8.50306	4.83373	64.7	30	6	0
Ifiang Nsung	Bakassi	8.54825	4.85058	50.2	30	4	0
Edikokon Idem	Bakassi	8.56455	4.83314	33.6	30	5	0
Akwa Ikot Eyo Edem	Akpabuyo	6.51564	4.90714	69.7	30	5	2
Oban	Akamkpa	8.58035	5.32022	133.3	30	4	0
Mfum1	Etung	8.84605	5.81277	95.5	30	4	4
Mfum2	Etung	8.84532	5.81332	97.6	30	5	0
Agbokun Carabot	Etung	8.89208	5.90468	133.7	30	5	2
Ajassor Plantation	Etung	8.87186	5.89488	170.1	30	5	0
Ajassor	Etung	8.85309	5.84296	90.6	30	1	0
Biajor	Boki	9.03124	6.00293	129.4	30	3	0
Ekumpour	Wula	9.1147	6.41078	176.5	30	4	0
Okwabang	Boki	9.12722	6.43939	199.6	30	4	0
Lishkwel bendi 11	Obanliku	9.16376	6.50821	183.1	30	4	0
Ketting	Obanliku	9.19799	6.57853	270.7	30	4	0
Sankwala	Obanliku	9.24167	6.61572	269.2	30	3	0
Belinge	Obanliku	9.44124	6.47229	444.5	30	1	0
Total					630	90	10

[a]LGA= Local government area; [b]NPS= Number of plants sampled; [c]NCLO= Number of cassava leaves obtained; [d]NOW= Number of weeds obtained

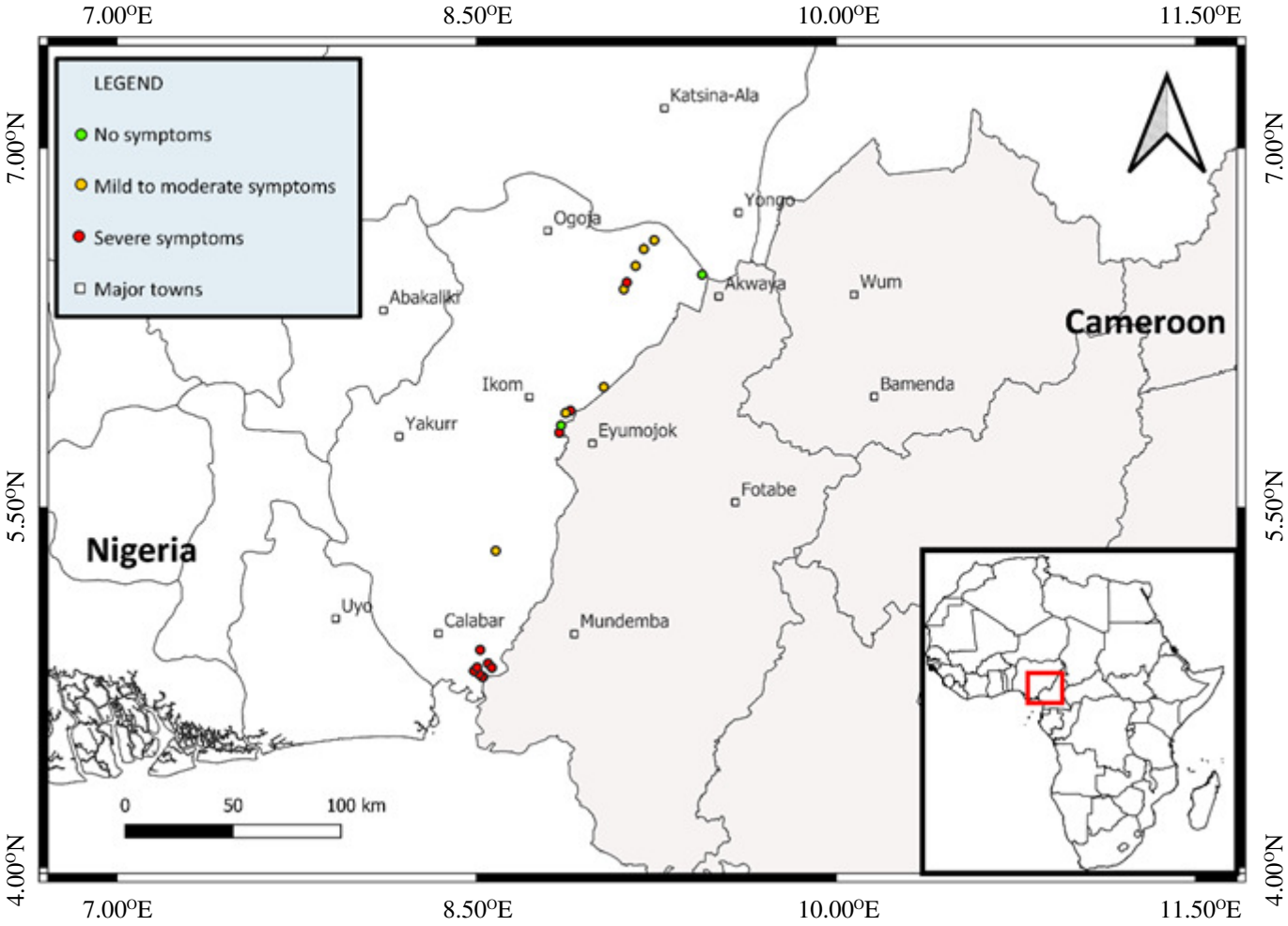

Figure 1. Distribution of leaf mosaic symptoms on cassava leaves and weeds along the Nigeria-Cameroon border.

were accessed for CMD incidence and severity assessment along two diagonal transects; 15 plants from each diagonal. Field CMD incidence was computed using the the formula of Rabindran (2011).

$$\text{Incidence} = \frac{\text{Total number of symptomatic plants}}{\text{Total number of plants sampled}} \text{ X } 100$$

Disease severity was assessed visually using the 1 to 5 rating scale (Hahn *et al.,* 1979). Figure 2 displays the pictorial representation of the rating scale.

Nucleic acid extraction. Cassava leaf samples were dried using an MD5 lyophilizer. The cetyltrimetylammonium bromide (CTAB) total nucleic acid extraction protocol optimised for cassava (Abarshi *et al.,* 2010) was adopted with slight modifications. Briefly, 100 mg of each dried cassava leaf was placed in microfuge tubes, and two free sterile steel balls were added. These were shaken on a TissueLyzer II (QIAGEN GmbH, D-40724 Hilden) at a frequency of 30 Hz for 90 seconds. This procedure was repeated once

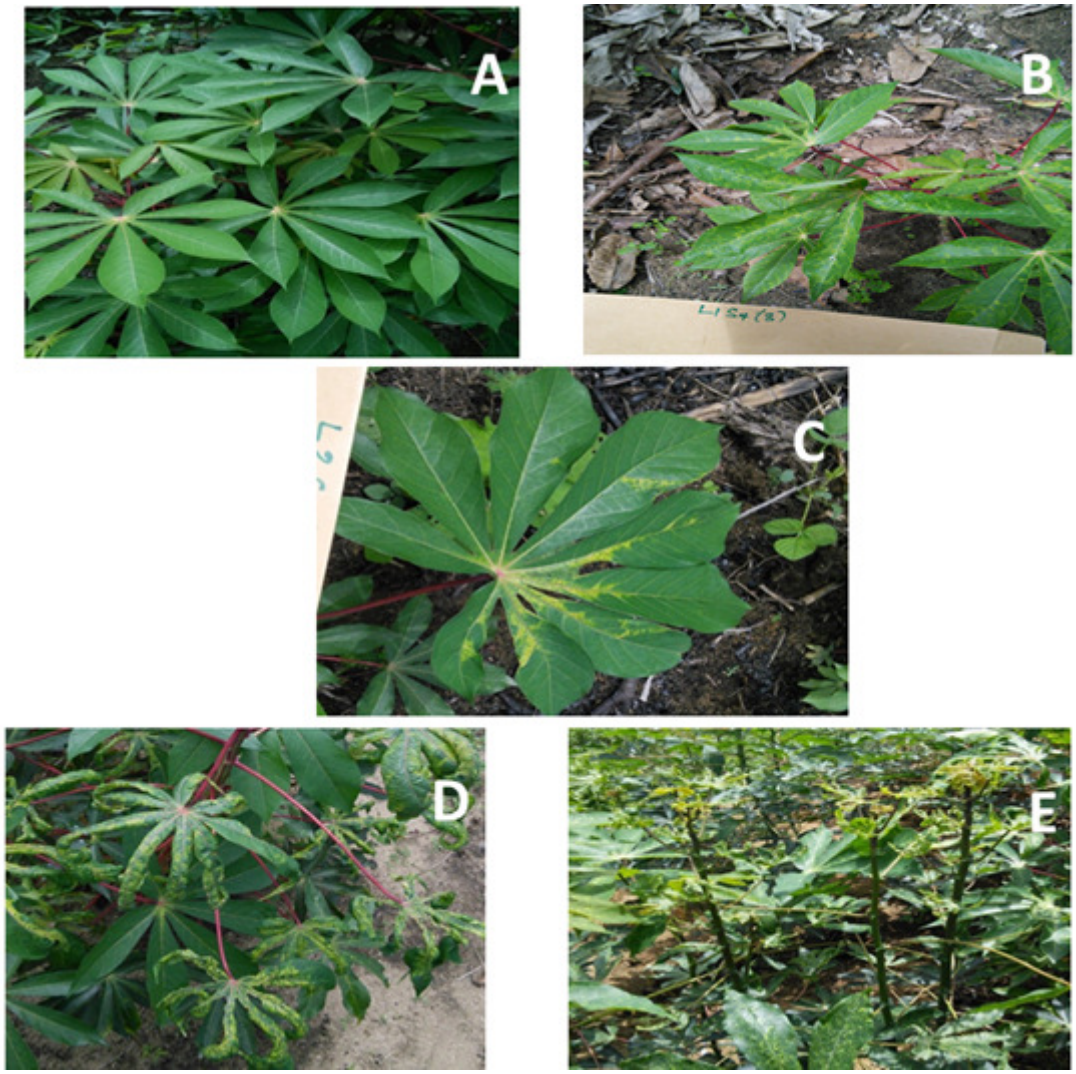

Figure 2. Severity scale to assess cassava mosaic disease symptoms. (A) Healthy, (B) slight mosaic, (C) mosaic, distortion and no decrease in leaf area, (D) severe mosaic, leaf distortion, curling and decrease in leaf size, and (E) most severe symptoms and stunting of plants.

to completely grind the leaf material into fine powder. Tubes with leaf powders were transferred into 2 ml microtubes and placed in a fume hood. Seven hundred microliters of CTAB buffer (2.0%, w/v CTAB, 2.0 M NaCl, 2.0% PVP, 25 mM EDTA, 100 mM Tris-HCl pH 8.0) were added. 2-mercaptoethanol (140 µl) was added to the mixtures and shaken vigorously on a vortex mixer. These were incubated in a water bath at 65 °C for 10 minutes.

The nucleic acids were extracted by adding 750 µl of phenol:chloroform: isoamylacohol mixture in a ratio of 25:24:1, vortexed and centrifuged at 10,000 *g* for 10 minutes. Then, 500 µl of the aqueous phase were transferred to new sterile 2 ml microfuge tubes.

The nucleic acids were precipitated in 300 µl of cold isopropanol, incubated at -20 °C for 1 hour, and pelleted by centrifuging at 10,000 *g* for 10 minutes. The supernatants were carefully decanted, while the pellets washed twice in 700 µl of 70% cold ethanol and centrifuged at 10,000 *g* for 5 minutes. The excess ethanol was completely removed by pipetting and air drying for 30 minutes at room temperature. The pellets were dissolved in 50 µl of nuclease-free sterile water, and stored in a -20 °C freezer until analysis. DNA integrity was determined by agarose gel electrophoresis.

Polymerase chain reaction. Total nucleic acid extracts from these samples were tested by PCR for the presence of ACMV and EACMV DNA (Alabi *et al.,* 2008), and the expected amplicon sizes were 368 bp and 650 bp, respectively. The primers used included ACMV (CMBRep-F, ACMVRep-R) and EACMV (CMBRep-F, EACMVRep-R), as shown in Table 2.

The reaction mixture per tube contained 1.25 µl of 10x Standard *Taq* reaction buffer consisting of 0.75 µl of $MgCl_2$ (25 mM), 0.25 µl of dNTPs (10 mM), 0.06 µl of *Taq* DNA polymerase (400 U), 0.25 µl mixture of CMBRep-F, ACMVRep-R and EACMVRep-R (10 µM for each primer), 7.94 µl of sterilised distilled water and 2.0 µl of each nucleic acid template. The cycling conditions for ACMV and EACMV were a preamplification phase consisting of one cycle of denaturation at 94 °C for 1 minute, annealing at 52 °C for 2 minutes and extension at 72 °C for 3 minutes. Amplification of the nucleic acid template was achieved *via* 35 cycles of denaturation at 94 °C for 1 minute, annealing at 52 °C for 2 minutes and extension at 72 °C for 1 minute 33 seconds, followed by a final extension at 72 °C for 5 minutes. The PCR-amplified products were resolved by 1.5% agarose gel electrophoresis and visualised under ultraviolet

TABLE 2. Primers used to detect African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV) by PCR in cassava and weed samples collected along the Nigeria-Cameroon border of Cross River State, Nigeria

Target virus[a]	Primer names	Sequences (5'-3')
ACMV	CMBRep-F	CRTCAATGACGTTGTACCA
	ACMVRep-R	CAGCGGMAGTAAGTCMGA
EACMV	CMBRep-F	CRTCAATGACGTTGTACCA
	EACMVRep-R	CRTCAATGACGTTGTACCA

The primers used were described by Alabi *et al*. (2008b), and the nucleotide letters 'R' and 'M' indicate 'A/G' and 'A/C', respectively

[a]ACMV= Africa cassava mosaic virus*;* EACMV= East Africa cassava mosaic virus

(UV) light using a gel documentation system. A 100 bp molecular weight marker (Promega, Madison, USA) was run in each gel as a reference to estimate the size of the virus-specific DNA band among the PCR amplified products.

RESULTS

Occurrence of CMD. Cassava mosaic disease symptoms were observed in locations as presented in Table 1. Only Ajassor and Belinge showed no symptoms of the disease. Varying degrees of symptom severity, which included mosaic, chlorotoc leaf distortion, leaf curling and stunting of plants, were observed across a range of the study sites (Fig. 2), with no specific distribution pattern of symptoms (Fig. 2).

Five weed species with mosaic and chlorotic symptoms were obtained from Ikang 1, Akwa Ikot Eyo Edem, Mfum 1 and Agbokun Carabot (Table 1). However, after testing for the presence of ACMV and EACMV, only *Sclerocarpus africanus* belonging to the family *Asteraceae* and sampled from Ikang 1 tested positive for EACMV (Fig. 3).

CMD incidence and severity. CMD incidence ranged from 0 to 100%, with the highest value recorded in Ifiang Nsung (L6). The lowest incidence value was observed at Ajassor (L14) and Belinge (L21) sampling sites (Fig. 4). In all 21 locations sampled, CMD severity was mild at ten sites (Fig. 5). Of the fields sampled, seven locations had generally moderate severity scores (2 and 4 rating scales); while severe symptoms were prevalent at four sites (Fig. 5).

Detection of viruses by PCR. Nucleic acid extracts from only four out of the 90 cassava and ten weed plants produced the expected amplicon size of 650 bp for EACMV alone (data not shown): three from cassava leaves (two symptomatic and one asymptomatic) and one from the weed *S. africanus* (Table 3). African cassava mosaic virus alone, with the expected amplicon size of 368 bp, was detected in nine

Figure 3. *Sclerocarpus africanus* plant that tested positive for East African cassava mosaic virus at Ikang 1 along the Nigeria-Cameroon border.

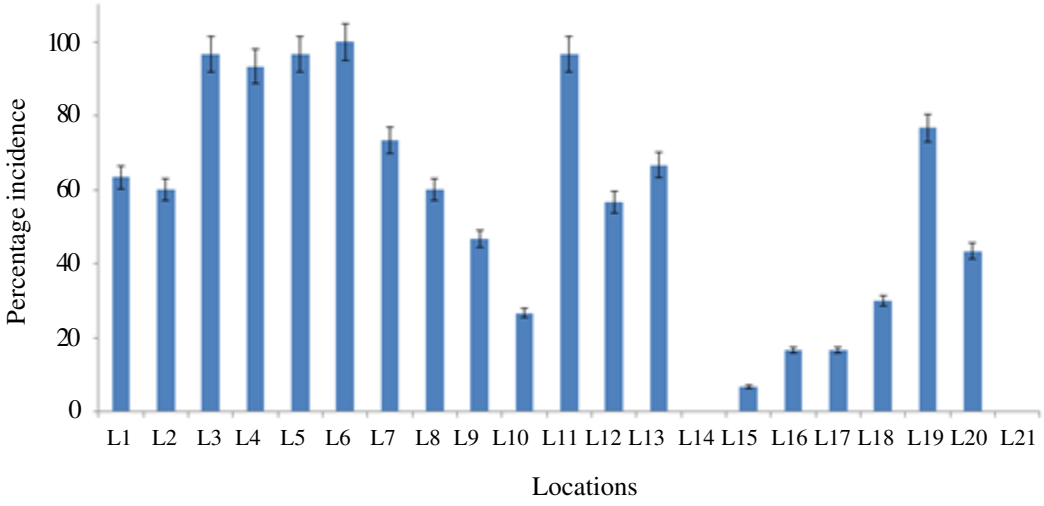

Figure 4. Incidence of cassava mosaic disease at sites along the Nigeria - Cameroon border of Cross River State, Nigeria. L1= Ikang 1, L2= Ikang 2, L3= Ikang 3, L4= Edik Idim Ikot Eyi, L5= Edik Idim Ikot Nsid, L6= Ifiang Nsung, L7= Edikokon Idem, L8= Akwa Ikot Eyo Edem, L9= Oban, L10= Mfum 1, L11= Mfum 2, L12= Agbokun carabot, L13= Ajassor plantation, L14= Ajassor, L15= Biajor, L16= Ekumpour, L17= Okwabang, L18= Lishkwel bendi 11, L19= Ketting, L20= Sankwala, L21= Belinge.

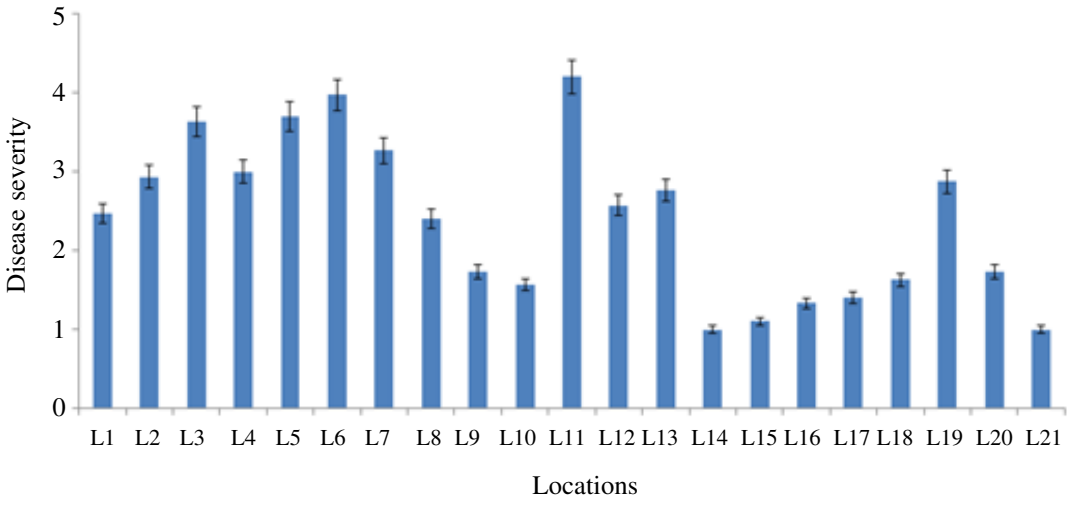

Figure 5. Mean severity of cassava mosaic disease along the Nigeria - Cameroon border in Cross River State, Nigeria. LI= Ikang 1, L2= Ikang 2, L3= Ikang 3, L4= Edik Idim Ikot Eyi, L5= Edik Idim Ikot Nsid, L6= Ifiang Nsung, L7= Edikokon Idem, L8= Akwa Ikot Eyo Edem, L9= Oban, L10= Mfum 1, L11= Mfum 2, L12= Agbokun carabot, L13= Ajassor plantation, L14= Ajassor, L15= Biajor, L16= Ekumpour, L17= Okwabang, L18= Lishkwel bendi 11, L19= Ketting, L20= Sankwala, L21= Belinge.

TABLE 3. Polymerase chain reaction results confirming single and mixed infections of African cassava mosaic virus and East African cassava mosaic virus in sampled plants

Location	LGA[a]	Plant	Number of samples		ACMV[d]		EACMV[e]		ACMV + EACMV	
			S[b]	NS[c]	S	NS	S	NS	S	NS
Ikang 1	Bakassi	Cassava	7	1	0	0	1	0	1	0
Ikang 1	Bakassi	Weed	2	0	0	0	1	0	0	0
Ikang 2	Bakassi	Cassava	3	1	0	0	0	0	2	1
Ikang 3	Bakassi	Cassava	5	1	1	0	0	0	1	0
Edik Idim Ikot Nsid	Bakassi	Cassava	4	1	0	0	0	0	1	0
Ifiang Nsung	Bakassi	Cassava	4	0	2	0	0	0	1	0
Edikokon Idem	Bakassi	Cassava	4	1	1	0	0	0	1	0
Akwa Ikot Eyo Edem	Akpabuyo	Cassava	4	1	0	0	0	1	2	0
Mfum 1	Etung	Cassava	3	1	1	0	0	1	1	0
Mfum 2	Etung	Cassava	4	1	0	1	0	0	0	0
Agbokun Carabot	Etung	Cassava	4	1	1	0	0	0	1	0
Ajassor plantation	Etung	Cassava	4	1	0	1	0	0	0	0
Ekumpour	Wula	Cassava	3	1	0	0	0	0	1	0
Belinge	Obanliku	Cassava	0	1	0	1	0	0	0	0
Total			51	12	6	3	2	2	12	1

[a]LGA = Local Government Area; [b]S = Symptomatic; [c]NS = Non Symptomatic; [d]ACMV = African cassava mosaic virus; [e]EACMV = East African cassava mosaic virus. Locations are listed in Table 1

samples, all from cassava leaves (Table 3). Thirteen samples indicated mixed infections of ACMV and EACMV, twelve from symptomatic and one from asymptomatic cassava leaves. None of the samples from Edik Idim Ikot Eyi, Oban, Ajassor, Biajor, Okwabang, Lishkwel bendi 11, Ketting or Sankwala tested positive for either ACMV or EACMV. Only one weed sample collected at Ikang 1 tested positive for EACMV; no other weed samples collected tested positive for either ACMV or EACMV.

DISCUSSION

Occurrence of CMD. There was widespread CMD occurrence, with moderate to high incidence in many areas along the Nigeria-Cameroon border (Table 3). This may be attributable to the use of highly susceptible local cultivars or CMD-affected planting materials. A recent study highlighted the extensive use of infected planting materials and high disease severity across cassava fields in eastern Nigeria, close to the Nigeria-Cameroon border region (Igwe *et al.,* 2021). Most plants showing severe symptoms were found to have single or mixed infections of ACMV and EACMV, confirming other earlier reports in Nigeria (Ariyo *et al.*, 2005) and Cameroon (Fondong *et al.*, 2000b). Although the severest symptoms were attributed to the synergistic effect of double infection (Fondong *et al.,* 2000a,b; Pita *et al.,* 2001), further investigations are required to determine the cause of the severe symptoms associated with single ACMV and EACMV infections that we observed.

The distribution of CMD within the border revealed no specific pattern as varying levels of symptoms were observed across the sampling sites (Fig. 5). This may be due to unrestricted movement of infected planting materials within the area, leading to exchange of diseased stems and then sporadic CMD occurrences and spread across the locations. There is need to for regulation of the exchange of cassava materials to limit the risk of virus spread along these areas for effective disease management.

Incidence and severity of CMD. Single infections of ACMV were found to predominate over EACMV infections within the Nigeria-Cameroon border (Table 3). Only 40% of the cassava samples yielded single infections of EACMV, which is consistent with the report of Ogbe *et al.* (2006), who investigated CMD causal agents in Nigeria. This could occur because natural single EACMV occurrences tend not to elicit maximum damage on cassava plants compared to mixed ACMV-EACMV infections (Alabi *et al.,* 2011). Single infections of EACMV were also not common in Cameroon (Fondong *et al.,* 2000a), which could explain why single EACMV infections were trace in the present study. Additionally, mixed ACMV and EACMV infections were more common than single infections of either ACMV or EACMV.

A recent study (Eni *et al*., 2020) also revealed the prevalence of both CMGs within a different geographical area in Nigeria. Another report on CMGs across agro-ecologies in Cross-River State showed similar trends (Ekpiken *et al.,* 2022).

The high distribution of mixed infections in some of the farms may be a reflection of uncontrolled movement of double-infected planting materials (Alabi *et al.,* 2011; Kolade *et al.,* 2022). Effective CMD management starts from the use of virus-free, certified cassava stems for cassava fields and a robust testing regime to detect CMG infections.

Detection of viruses by PCR. Five non-symptomatic cassava leaf samples tested positive to ACMV and EACMV DNA *via* PCR (Table 3), thus confirming the limitations of visual field assessments for CMG infections especially among newly infected plants. This exposes the inherent dangers of latent CMG infections as future potential sources of CMD along the Nigeria-Cameroon border region.

This is consistent with the findings of Ogbe *et al.* (2006), who also identified CMGs in asymptomatic cassava leaves. It thus favours the certification of cassava planting materials. Symptomatic plants that tested negative by PCR for either ACMV or EACMV may be a result of cassava green mite infestation symptoms, which can mimic those of CMD (Samura *et al*., 2014). Further studies are important to determine virus diversity along the Nigeria-Cameroon border, for example, by high-throughput sequencing, which has the capability to reveal unknown, emerging, or invasive viruses in the region (Wamonje, 2020).

The positive detection of EACMV in one of the weeds (*S. africanus*) from Ikang 1 tested confirms previous reports that weeds serve as reservoirs of these viruses (Alabi *et al.,* 2008a). This is the first report to suggest that *S. africanus* serves as an alternative host of ACMV. Since infected weeds are potential CMG reservoirs, this information is of epidemiological significance for the effective management of CMD in cassava in SSA.

The occurrence of uninfected cassava plants observed in some of study sites (Table 3) may be due to the distribution and use of healthy, resistant, and/or tolerant cassava cultivars by farmers in the areas (Manyong *et al.,* 2000). This therefore, implies that increasing farmers' awareness of the cause and nature of the disease and possible control measures will bolster their participation in the management and possible eradication of the disease within the borders of Nigeria. The control of CMD is achieved mainly through use of resistant or tolerant cultivars, employing adequate phytosanitary techniques to destroy diseased plants and use of healthy stems (Legg *et al.,* 2015).

There could be chances of emergence of new strains/variants of the causative agents due to the frequent reports of dual/multiple infections (Fondong *et al.,* 2000). Constant surveillance, phytosanitation, and improvement of existing diagnostics will continue to play major roles in ensuring that Nigeria retains its non-epidemic status and low epidemic index. It is also important to ensure that existing quarantine measures continue to be enforced.

CONCLUSION

Cassava mosaic disease is an important biotic constraint to cassava production along the Nigeria-Cameroon border and the major CMG is ACMV; while EACMV occur at a lower frequency. There is a need for further efforts to control CMD though improved phytosanitary measures and implementation of extension programs. The deployment of improved varieties and PCR-tested propagation stocks, weed control and phytosanitary techniques for destroying diseased plants, together with improved farmer education on cassava diseases and countermeasures, remain crucial for CMD management. There is urgent need to determine other alternate hosts, apart from the previously identified hosts, that can also serve as virus reservoirs. Further studies are necessary to determine virus diversity *via* sequencing for more in-depth knowledge on viruses associated with CMD and their alternate hosts along the Nigeria–Cameroon borders.

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REFERENCES

Abarshi, M.M., Mohammed, I.U., Wasswa, P., Hillocks, R.J., Holt, J., Legg, J.P., Seal, S. E. and Maruthi, M.N. 2010. Optimization of diagnostic RT-PCR protocols and sampling procedures for the reliable and cost-effective detection of *Cassava brown streak virus*. *Journal of Virological Methods* 163:353-359.

Alabi, O.J., Ogbe, F.O., Bandyopadhyay, R., Dixon, A.G.O., Hughes, J.d'A. and Naidu, R.A. 2007. The occurrence of *African cassava mosaic virus* and *East African cassava mosaic Cameroon virus* in natural hosts other than cassava in Nigeria. Phytopathology 97(7):S3-S3.

Alabi, O.J., Ogbe, F.O., Bandyopadhyay, R., Kumar, P.L., Dixon, A.G.O., Hughes, J.d'A. and Naidu, R.A. 2008a. Alternate hosts of *African cassava mosaic virus* and *East African cassava mosaic Cameroon virus* in Nigeria. *Archives of Virology* 153:1743-47.

Alabi, O.J., Kumar, P.L. and Naidu, R.A. 2008b. Multiplex PCR for the detection of *African cassava mosaic virus* and *East African cassava mosaic Cameroon virus* in cassava. *Journal of Virological Methods* 154(1-2):111-120.

Alabi, O.J., Kumar, P.L. and Naidu, R.A. 2011. Cassava mosaic disease: A curse to food security in sub-Saharan Africa. https://www.apsnet.org/edcenter/apsnetfeatures/Pages/cassava.aspx. Accessed 10 November 2021.

Ariyo, O.A., Koerbler, M., Dixon, A.O., Atiri, G.I. and Winter, S. 2005. Molecular variability and distribution of cassava mosaic begomoviruses in Nigeria. *Journal of Phytopathology* 153:226-231.

Bock, K.R. and Woods, R.D. 1983. Etiology of African cassava mosaic disease. *Plant Disease* 67(9):994-995.

Ekpiken, E.E., Eyong, O.I. and Adediji, A.O. 2022. Occurrence and molecular characterization of begomoviruses associated with cassava growing regions of Cross River State, Nigeria. *Journal of Applied Horticulture* 24(1):9-15.

Eni, A.O., Efekemo, O.P., Onile-ere, O.A. and Pita, J.S. 2020. South West and North Central Nigeria: Assessment of cassava mosaic disease and field status of *African cassava mosaic virus* and *East African cassava mosaic virus. Annals of Applied Biology* 178:466-479.

Fondong, V.N., Pita, J.S., Rey, M.E.C., de Kochko, A., Beachy, R.N. and Fauquet C.M. 2000a Evidence of synergism between *African cassava mosaic virus* and a new double-recombinant geminivirus infecting cassava in Cameroon. *Journal of General Virology* 81:287-297.

Fondong, V.N., Thresh, J.M. and Fauquet, C. 2000b. Field experiments in Cameroon on *Cassava mosaic virus* disease and the reversion phenomenon in susceptible and resistant cassava cultivars. *International Journal of Pest Management* 46:211-217.

Hahn, S.K., Terry, E.R., Leuschner, K., Akobundu, I.O., Okali, C. and Lal, R. 1979. Cassava improvement in Africa. *Field Crops Research* 2:193-226.

Igwe, D.O., Anyanwu, C.B., Afiukwa, C.A., Nnamani, C.V., Nweke, F.N., Ude, G.N. and Ubi, B.E. 2001. Phenotypic and molecular screenings for determination of cassava mosaic disease (CMD) status in farmers' fields in Ebonyi State, Nigeria. *Molecular Biology Reports* 48:227-240.

Kolade, O.A., Popoola, A.R., Igwe, D.O. and Adediji, A.O. 2022. Molecular diagnostics for plant viruses towards enhanced delivery of disease-free planting materials and germplasm exchange. In: *Agricultural*

Biotechnology, Biodiversity and Bioresources Conservation and Utilization. Obembe, O.O, Ekundayo, E.O., Okoli, A.S., Gidado, A., Adetunji, C.O., Ibrahim, A.B. and Ubi, B.E. (Eds.). CRC Press, Taylor and Francis Group. Vol 2, Number 10. pp. 179-200.

Legg, J.P., Kumar, P.L., Makesh Kumar, T., Tripathi, L., Ferguson, M., Kanju, E., Ntawuruhunga, P. and Cuellar, W. 2015. Cassava virus diseases: Biology, epidemiology and management. *Advances in Virus Research* 91:85-142.

Manyong, V.M., Dixon, A.G.O., Makinde, K.O., Bokanga, M. and Whyte, J. 2000. The contribution of IITA-improved cassava to food security in sub-Saharan Africa: An impact study. International Institute of Tropical Agriculture (IITA), Ibadan. Nigeria. pp. 1-13.

Ndunguru, J., Legg, J.P., Aveling, T.A.S., Thompson, G. and Fauquet, C.M. 2005. Molecular biodiversity of *Cassava Begomovirus* in Tanzania: Evolution of cassava geminiviruses in Africa and evidence for East Africa being a center of diversity of cassava geminiviruses. *Virology Journal* 2:21-44.

Nweke, F.I., Dixon, A.G.O., Asiedu, R. and Folayan S.A. 1994. Cassava - varietal needs of farmers and the potential for production growth in Africa. IITA Ibadan, Nigeria. 239 pp. https://hdl.handle.net/10568/97295. Accessed 31 April 2022.

Ogbe, F.O. 2001. Survey of cassava begomoviruses in Nigeria and the response of resistant cassava genotypes to African cassava mosaic begomoviruses infection. Ph.D. Thesis. University of Ibadan, Nigeria. 166pp.

Ogbe, F.O., Thottappilly, G., Dixon, A.G.O., Atiri, G.I. and Mignouna, H.D. 2003. Variants of *East African cassava mosaic virus* and its distribution in double infections with *African cassava mosaic virus* in Nigeria. *Plant Disease* 87:229-232.

Ogbe, F.O., Dixon, A.G.O., Hughes, J. d'A., Alabi, O.J. and Okechukwu R.O. 2006. Status of cassava begomoviruses and their new natural hosts in Nigeria. *Plant Disease* 90:548-553.

Owor, B., Legg, J.P., Okao-Okuja, G., Obonyo, R. and Ogenga-Latigo, M.W. 2002. Interactions between cassava mosaic geminiviruses and the African pandemic of cassava mosaic disease. In: *Proceedings of First Integrated Pest Management Conference for sub-Saharan Africa,* 8-12 September, 2002. pp. 355-361. Makerere University, Kampala, Uganda. https://hdl.handle.net/10568/109330. Accessed 02 January 2023.

Pita, J.S., Fondong, V.N., Sangare, A., Otim-Nape, G.W., Ogwal, S. and Fauquet, C.M. 2001. Recombination, pseudo-recombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. *Journal of General Virology* 82: 655-665.

Rabindran, R. 2011. Survey for the occurrence of cassava mosaic disease in Tamil Nadu. *Journal of Root Crops* 37:197-199.

Samura, A.E., Massaquoi, F.B., Mansaray, A. Kumar, P.L., Koroma, J.P.C., Fomba, S.N. and Dixon, A.G.O. 2014. Status and diversity of the cassava mosaic disease causal agents in Sierra Leone. *International Journal of Agriculture and Forestry* 4: 246-254.

Saokham, K., Hemniam, N., Roekwan, S., Hunsawattanakul, S., Thawinampan, J. and Siriwan W. 2021. Survey and molecular detection of Sri Lankan cassava mosaic virus in Thailand. *PLoS One* 16(10): e0252846.

Sseruwagi, P., Sserubombwe, W.S., Legg, J.P., Ndunguru, J. and Thresh, J.M. 2004. Methods of surveying the incidence and severity of cassava mosaic disease and whitefly vector populations on cassava in Africa: A review. *Virus Research* 100:129-142.

Thresh, J.M., Fargette, D. and Otim, N. 1994. The viruses and virus diseases of cassava in Africa. *African Crop Science Journal* 2: 459-478.

Umunakwe, P.C., Nwakwasi, R.N., Ani, A.O., Ejiogu-Okereke, E.N. and Nnadi, F.N. 2015. Constraints to the adoption of improved cassava varieties among rural farmers in Imo State, Nigeria. *Asian Journal of Agricultural Extension Economics and Sociology* 6:56-63.

Wamonje, F.O. 2020. Post-COVID-19 action: Guarding Africa's crops against viral epidemics requires research capacity building that unifies a trio of transdisciplinary interventions. *Viruses* 12(11):1276-1289.