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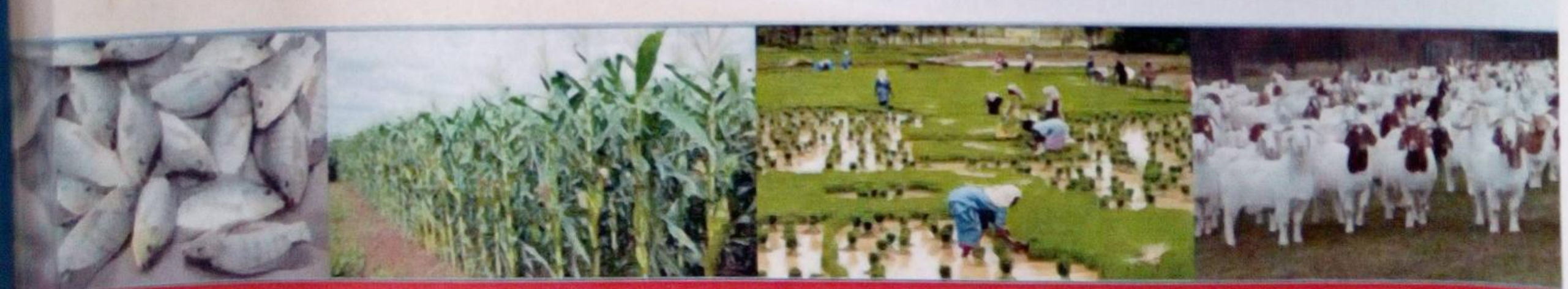

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MOLECULAR IDENTIFICATION OF VIRUSES INFECTING CASSAVA IN SELECTED LOCAL GOVERNMENT AREAS OF OYO STATE, NIGERIA

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ABSTRACT

Cassava is one of the most important food crops in Nigeria in terms of production and utilization. However, low productivity of the crop is associated with major virus diseases. A survey was conducted to identify the viruses infesting cassava in five Local Government Areas (LGAs) of Oyo State Nigeria. Twenty-five cassava fields were surveyed in February 2018 during which 75 symptomatic cassava leaves were collected. Total nucleic acid extraction of the samples was done using Cetyl Trimethyl Ammonium Bromide (CTAB) extraction protocol followed by multiplex Polymerase Chain Reaction (PCR) for African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV) identification. Symptoms observed included leaf curling, mosaic and "candle-like" sticks. Both ACMV and EACMV were detected in single infections at Akinyele LGA, whereas only ACMV was found in Atiba, Egbeda and Ikereku LGAs. Conversely, none of the samples from Afijio LGA tested positive for both viruses. Of the total leaf samples, 22.7 % were positive for ACMV, whereas 2.7 % tested positive for EACMV. The highest ACMV disease incidence was encountered in Akinyele (6.7 %), Egbeda (6.7 %) and Ikereku (6.7 %) LGAs while 2.7 %-of the total samples were positive for ACMV in Atiba LGA. The low incidences of ACMV and EACMV implied that cassava production is not threatened by these viruses in the surveyed areas. However, adoption of resistant cassava cultivars should be intensified as a precautionary measure.

KEYWORDS: African cassava mosaic virus, Disease incidence, East African cassava mosaic virus, Polymerase chain reaction, Survey

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) originated in South America but is an important root crop in sub-Saharan Africa. In Nigeria, it is grown in all agro-ecological zones and plays a vital role towards food security. In 2016, about 277.1 million tonnes of cassava were produced worldwide (FAO, 2016). In Africa, its production stood at 157.3 million tonnes. The top ten cassava producing countries were Nigeria (57.1 million tonnes), Thailand (31.2 million tonnes), Brazil (21.1 million tonnes), Indonesia (20.7 million tonnes), Ghana (17.8 million tonnes), Democratic Republic of Congo (14.7 million tonnes), Viet Nam (11 million tonnes), Cambodia (10.2 million tonnes), Angola (9.9 million tonnes) and Mozambique (9.1 million tonnes). Besides food for human consumption, cassava is used for several other domestic and industrial purposes in sub-Saharan Africa. It can be processed into *gari*, *lafun*, starch, bread or boiled with beans (Samura *et al*., 2014). Low cassava yield has been attributed to several factors. It is mostly cultivated by smallholder resource poor farmers. In addition, insect pests and diseases are responsible for low output. In addition to *African cassava mosaic virus* (ACMV), several species of *Begomovirus* viruses inducing severe cassava yield losses have been confirmed. These are *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), *East African cassava mosaic Kenya virus* (EACMKV), *African cassava mosaic Burkina Faso virus* (ACMBFV), *Cassava mosaic Madagascar virus* (CMMV) and *Cassava brown streak virus* (CBSV) (Bull *et al*., 2006; Tiendrébéogo *et al*., 2012).

Single and mixed infections of ACMV and EACMV occur in nature. For instance, while Eni and Fasasi (2013) reported sole infection of ACMV in Southern Nigeria, dual infections of ACMV and EACMV has been reported in Nigeria, Sierra Leone, Tanzania and Zambia (Harrison *et al*., 1997; Chikoti *et al*., 2013; Ogbe *et al*., 2003; Samura *et al*., 2014). *Begomoviruses* are transmitted by various species of whitefly (*Bemisia tabaci* Genn.). However, in addition to virus transmission, whiteflies cause injury to cassava through direct sucking of fluid from the cells of infested plants. Cassava mealybug (*Phenacoccus manihoti*) and cassava green mite (*Mononychellus tanajoa*) are also economic pests that cause severe damage to cassava (Évila *et al*., 2012;

Yonow *et al.*, 2017). *African cassava mosaic virus* and *East African cassava mosaic virus* in singly infected plants can account for 100 % yield loss depending on virus strain and susceptibility of the cassava variety. Partial control of these viruses can be achieved through application of insecticides to suppress their insect vectors. However, adoption of resistant cultivars is the most sustainable strategy. The objective of this study was to determine the incidence and distribution of viruses infecting cassava in selected Local Government Areas of Oyo State, Nigeria.

METHODOLOGY

Survey and Sample Collection

Five Local Government Areas (Afijio, Akinyele, Atiba, Egbeda and Ikereku) of Oyo State were surveyed. Oyo State is located in the Southern part of Nigeria where a lot of cassava is cultivated. The survey was conducted in February, 2018 in popular cassava growing communities. In each Local Government Area (LGA), five cassava fields were visited. Leaf samples were collected from symptomatic cassava plants for ACMV and EACMV indexing. Three samples were collected from each field, making 15 samples in one LGA and a total of 75 samples from the five LGAs. The coordinates of each farm were recorded using Geographical Positioning System (GPS) device. Source of planting materials where available, size of the field, crops in adjacent fields and cropping history of each field were recorded. Symptomatic samples were detached from cassava stem and stored in the refrigerator at the temperature of 4 °C to avoid degradation pending the time of total nucleic acid extraction.

Total Nucleic Acid Extraction

The leaf samples were analyzed at the Virology and Molecular Diagnostics Laboratory, International Institute of Tropical Agriculture (IITA), Ibada, Oyo State. Samples were macerated at the rate of 0.50 mg mL^{-1} of Cetyl Trimethyl Ammonium Bromide (CTAB) DNA extraction buffer containing monothioglycerol (1 µL/mL) in sterile mortars and pestles (Kumar, 2009). The extract was transferred into 1.5 mL eppendorf tubes and incubated in waterbath at 65 °C for 10 minutes. Tubes were brought to room temperature and 600 µL of phenol, chloroform and isoamyl in the ratio of 25:24:1 was added. The tubes were vortexed and centrifuged at 12000 g for 10 minutes. Thereafter, 450 µL of the supernatant was carefully transferred into new autoclaved 1.5 mL eppendorf tubes and 2/3 volumes of cold isopropanol/isopropyl alcohol was added to the supernatant. The contents were mixed gently, incubated for one hour at -20 °C and then centrifuged at 12000 g for 10 minutes in order to sediment the nucleic acid. After this, the supernatant was carefully decanted. Then 500 µL of 70 % ethanol was added to the pellets and centrifuged at 12000 g for five minutes. The ethanol was carefully decanted and the DNA pellet was dried at 37 °C for 15 minutes. Thereafter, the DNA pellet was suspended in 50 µL of sterile distilled water and stored at -20 °C for further laboratory analysis.

Quantification of Extracted Samples and Integrity Testing by Agarose Electrophoresis

Sample quantification was done using 1 µL of the suspended DNA stock with a NanoDrop spectrophotometer (Model 2000, Thermoscientific). DNA concentration was determined; protein purity was quantified at 260/280 and other impurities at 260/230. Integrity (quality) of the quantified samples was analysed using 5 µL of the DNA stock mixed with 3 µL of loading dye in a loading plate. Then, 7 µL of the mixed sample was run on ethidium bromide stained 1.5 % agarose gel in tris acetic ethylene diamine tetra acetic acid (EDTA) buffer (TAE buffer) at 120 V for 50 minutes. The result was obtained by exposing the gel to ultraviolet light (UV) using BIO RAD Gel Doc EZ imager.

RESULTS AND DISCUSSION

Cassava was cultivated on 2 – 3 hectares in most of the fields visited. Symptoms such as leaf chlorosis, mosaic, curling and stunting were observed. The mosaic symptoms observed are in agreement with those reported by Samura *et al.* (2014) in some cassava producing zones of Sierra Leone. The diverse symptoms are in consonant with the observations of Patil and Fauquet (2009) who stated that different cassava viruses elicit different symptoms in susceptible cassava varieties. Whiteflies (*B. tabaci*) and cassava green mites were also rampant in most fields. The prevalence of whiteflies observed in this study agreed with the result obtained by Chikoti *et al.* (2015) in cassava mosaic disease (CMD) infected farms in Zambia. Whiteflies are important insects associated with numerous economic crops and are capable of transmitting several viruses including *Cassava mosaic virus*.

The incidence of CMD was generally low in all the surveyed locations. Of the 75 samples collected, five (6.7 %) from Akinyele LGA tested positive for ACMV (Fig. 1 and Table1). These samples were collected from Forobi, Ehin Oke and Otun Agbaakin. Within Akinyele LGA, three (4 %) samples collected from Ehin Oke were found positive for ACMV; one

(1.3 %) sample each was positive for ACMV at Forobi and Otun Agbaakin. Additionally, two (2.7 %) samples collected from Akinyele LGA were positive for EACMV and both were collected from Ehin Oke community (Fig. 1 and Table 1). The location (Ehin Oke) where both ACMV and EACMV were found is one of the major cassava-producing areas in Akinyele LGA. The incidence of the viruses could partly be attributable to the sources of planting materials. Based on the interview conducted during the survey, some of the farmers did not cultivate improved cassava varieties. Occurrence of ACMV and EACMV in the same location, as observed in the present study is consistent with the findings of Ogbe *et al.* (2006) during an extensive survey of some cassava growing States in Nigeria.

None of the samples from Afijio LGA tested positive for ACMV and EACMV (Table1). This could be attributable to the level of adoption of improved cassava varieties. Thus, all the farmers interviewed confirmed that they normally obtained planting materials from the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State. In Atiba LGA, two (2.7 %) samples tested positive for ACMV only and both samples were collected from Soro Osunyin (Fig. 1 and Table 1). In Egbeda LGA, five (6.7 %) samples tested positive for ACMV and all of them were collected from Egbeda community. Similarly, in Ikereku LGA, five (6.7 %) samples tested positive for ACMV. In this LGA, the highest incidence of ACMV was found at Odebode which had three (4 %) ACMV positive samples while two (2.7 %) samples were positive at Aba Oluode (Table 1). Some of the cassava plants that elicited reduced leaf size, mosaic and "candle stick: tested negative to ACMV and EACMV, suggesting that the symptoms were possibly induced by insect vectors or other pathogens (Chikoti *et al.*, 2015).

CONCLUSION AND RECOMMENDATIONS

This study revealed low incidence and distribution of ACMV and EACMV in Akinyele LGA, ACMV alone in three LGAs (Atiba, Egbeda and Ilkereku) and none in Afijio LGA. Inspite of the low occurrence of these viruses, adoption of resistant cultivars should be intensified by cassava farmers as a precautionary measure against the diseases.

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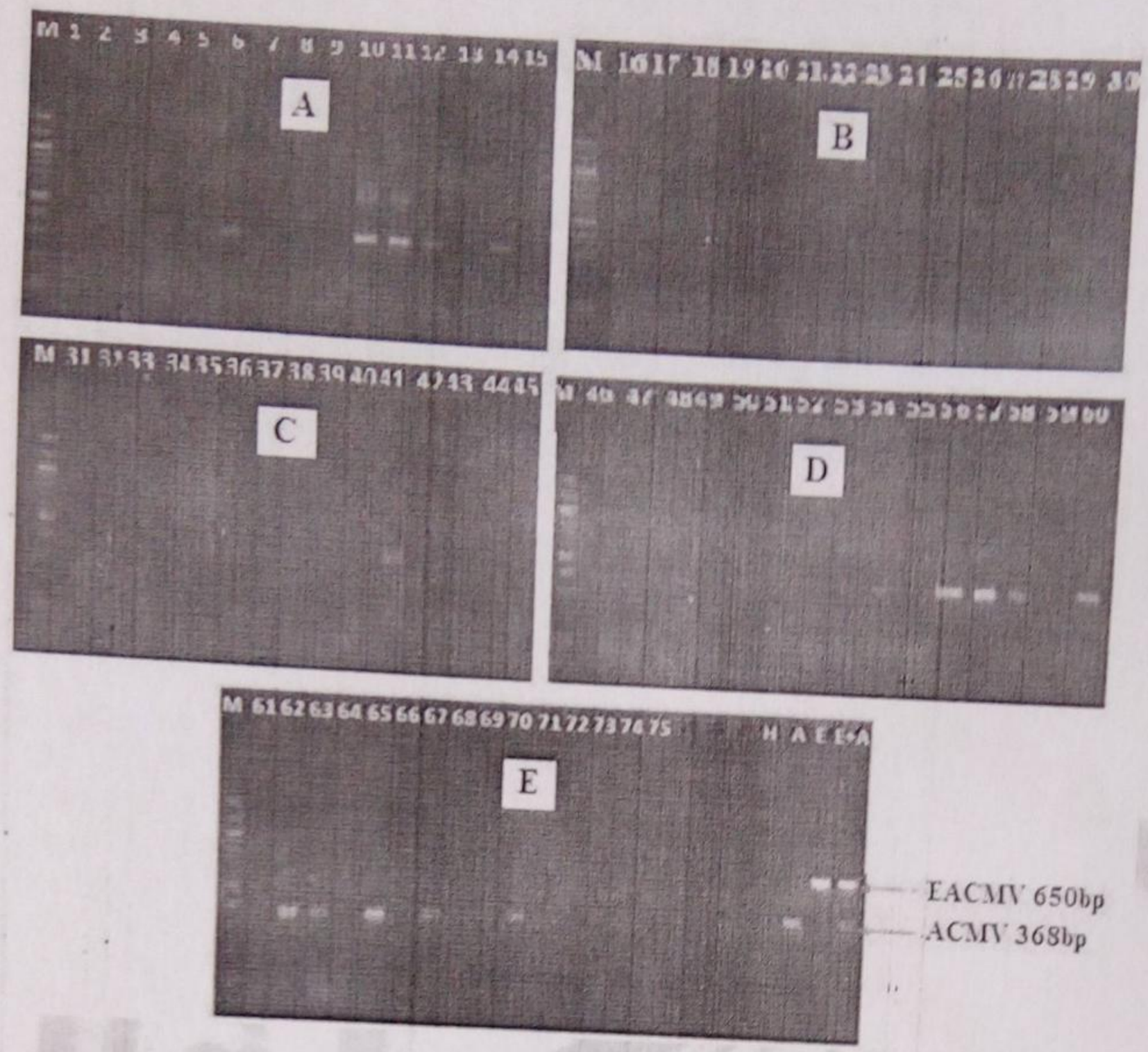

Fig. 1: Gel picture of the *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV) bands from cassava leaves sampled from (A) Akinyele, (B) Afijio, (C) Atiba, (D) Egbeda and (E) Ikereku Local Government Areas of Oyo State

1 -75 = Leaf samples ID from the various locations

Table 1: Incidence of *African cassava mosaic virus* and *East African cassava mosaic virus* in selected Local Government Areas (LGAs) of Oyo State, Nigeria in 2018

LGA	Location	Longitude (^{0}E)	Latitude (^{0}N)	Altitude (masL)	Virus Primer	
					ACMV	EACMV
Akinyele	Aba odo	3.91747	7.56951	267	-	-
	Aba odo	3.91747	7.56951	267	-	-
	Aba odo	3.91747	7.56951	267	-	-
	Forobi	3.92600	7.57077	268	-	-
	Forobi	3.92600	7.57077	268	-	-
	Forobi	3.92600	7.57077	268	+	-
	Ehin Oke	3.92567	7.50066	289	-	-
	Ehin Oke	3.92567	7.50066	289	-	-
	Ehin Oke	3.92567	7.50066	289	-	-
	Ehin Oke	3.92543	7.50045	213	+	+
	Ehin Oke	3.92543	7.50045	213	+	+
	Ehin Oke	3.92543	7.50045	213	+	-
	Otun Agbaakin	3.92442	7.50144	225	-	-
	Otun Agbaakin	3.92442	7.50144	225	+	-
	Otun Agbaakin	3.92442	7.50144	225	-	-
Afijio	Alaaka	3.91528	7.79877	305	-	-
	Alaaka	3.91528	7.79877	305	-	-
	Alaaka	3.91528	7.79877	305	-	-
	Jobele	3.91719	7.76487	272	-	-
	Jobele	3.91719	7.76487	272	-	-
	Jobele	3.91719	7.76487	272	-	-
	Jobele	3.91587	7.76104	284	-	-
	Jobele	3.91587	7.76104	284	-	-
Healthy control					-	-
ACMV Disease control					-	-
EACMV Disease control					+	-
ACMV+ EACMV Diseased control					-	+++
masl=metres above sea level					+	++

Table 1 Continued: Incidence of *African cassava mosaic virus* and *East African cassava mosaic virus* in selected Local Government Areas (LGAs) of Oyo State, Nigeria in 2018

LGA	Location	Longitude (°E)	Latitude (°N)	Altitude (masL)	Virus Primer	
					ACMV	EACMV
Afijio	Jobele	3.91587	7.76104	284	-	-
	Jobele	3.91171	7.74915	291	-	-
	Jobele	3.91171	7.74915	291	-	-
	Jobele	3.91171	7.74915	291	-	-
	Alaaka	3.91189	7.92740	284	-	-
	Alaaka	3.91189	7.92740	284	-	-
Atiba	Obakayeja	3.91130	7.92740	281	-	-
	Obakayeja	3.91130	7.92740	281	-	-
	Obakayeja	3.91130	7.92740	281	-	-
	Obakayeja	3.91189	7.92740	284	-	-
	Obakayeja	3.91189	7.92740	284	-	-
	Obakayeja	3.91189	7.92740	284	-	-
	Obakayeja	3.91116	7.92664	276	-	-
	Obakayeja	3.91116	7.92664	277	-	-
	Obakayeja	3.91116	7.92664	278	-	-
	Soro Osunyin	3.92292	7.92123	262	-	-
	Soro Osunyin	3.92292	7.92123	262	+	-
	Soro Osunyin	3.92292	7.92123	262	-	-
	Soro Osunyin	3.93550	7.87220	289	-	-
	Soro Osunyin	3.93550	7.87220	289	-	-
	Soro Osunyin	3.93550	7.87220	289	+	-
Egbeda	Iyana Ajia	3.92000	7.55608	254	-	-
	Iyana Ajia	3.92000	7.55608	254	-	-
	Iyana Ajia	3.92000	7.55608	254	-	-
	Iyana Ajia	3.92033	7.55662	258	-	-
	Iyana Ajia	3.92033	7.55662	258	-	-
	Iyana Ajia	3.92033	7.55662	258	-	-
	Egbeda	3.92043	7.55669	256	-	-
	Egbeda	3.92043	7.55669	256	-	-
Healthy control					-	-
ACMV Disease control					+	-
EACMV Disease control					-	+++
ACMV+ EACMV Disease control					+	++

masl=metres above sea level

Table 1 Continued: Incidence of *African cassava mosaic virus* and *East African cassava mosaic virus* in selected Local Government Areas (LGAs) of Oyo State, Nigeria in 2018

LGA	Location	Longitude (^{0}E)	Latitude (^{0}N)	Altitude (masL)	Virus Primer	
					ACMV	EACMV
Egbeda	Egbeda	3.92043	7.55669	256	+	-
	Egbeda	3.92102	7.55624	252	-	-
	Egbeda	3.92102	7.55624	252	+	-
	Egbeda	3.92102	7.55624	252	++	-
	Egbeda	3.92013	7.55639	259	+	-
	Egbeda	3.92013	7.55639	259	-	-
	Egbeda	3.92013	7.55639	259	+	-
Ikereku	Odebode	3.92798	7.57683	291	-	-
	Odebode	3.92798	7.57683	291	++	-
	Odebode	3.92798	7.57683	291	+	-
	Odebode	3.92756	7.57612	288	-	-
	Odebode	3.92756	7.57612	288	++	-
	Odebode	3.92756	7.57612	288	-	-
	Aba Oluode	3.92553	7.57551	300	+	-
	Aba Oluode	3.92553	7.57551	300	-	-
	Aba Oluode	3.92553	7.57551	300	-	-
	Aba Oluode	3.92782	7.57674	292	+	-
	Aba Oluode	3.92782	7.57674	292	+	-
	Aba Oluode	3.92782	7.57674	292	-	-
	Olukitibi	3.92727	7.57214	272	-	-
	Olukitibi	3.92727	7.57214	272	-	-
	Olukitibi	3.92727	7.57214	272	-	-
Healthy control					-	-
ACMV Disease control					+	-
EACMV Disease control					-	+++
ACMV+ EACMV Disease control					+	++

masl=metres above sea level

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