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INCIDENCE AND DISTRIBUTION OF VIRUSES INFECTING CASSAVA IN KADUNA AND SOKOTO STATES, NIGERIA

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ABSTRACT

Field survey was conducted in the 2015 wet season to determine the incidence and distribution of cassava viruses in Kaduna and Sokoto States, Nigeria. Eighteen farms from 3 Local Government Areas namely; Lere, Chikun and Kajuru (Kaduna State) and Tureta, Shagari and Tambuwal (Sokoto State) were surveyed. Symptomatic leaves (180) and asymptomatic leaves (90) were collected from the surveyed farms. Enzyme Linked Immosorbent Assay (ELISA) technique was used to test the presence of viruses infecting cassava. Three viruses: *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV) and *Cassava Congo sequivirus* (no acronyms) were detected. Triple Antibody Sandwich (TAS- ELISA) was used to detect ACMV and EACMV while Double Antibody Sandwich (DAS- ELISA) was used to detect *Cassava Congo sequivirus*. ACMV had the highest incidence and was widely spread, the highest incidence was recorded in Lere Local Government (68.9%) and least was Tambuwal LG (11.0), followed by EACMV with highest incidence in Tambuwal (22.23%) and no occurrence in Kajuru. *Cassava Congo sequivirus* recorded the highest incidence in Kajuru (43.33%). Mixed infections occur in some of the farms. This the first report of *Cassava Congo sequivirus* in Nigeria and Africa. Wide spread occurrence of ACMV calls for effective management of the virus.

Keywords: Viruses, occurrence, cassava and survey

INTRODUCTION

Cassava, *Manihot esculenta* Crantz, is a shrubby perennial plant grown mainly for its carbohydrate rich tuberous roots (Thresh and Cooter, 2005). It is a widely cultivated crop of the genus *Manihot* (Fauguet and Fargette, 1990). It is among the leading food crops of the world (Nassar, 2007). It is an important crop in Nigeria which serves as food and income generation. Nigeria, is the largest producer of cassava in the world, producing 57.8 MT in 2016 (FAOSTAT, 2017). The optimum growth and performance of cassava in Guinea and Sudan Savannah is threatened by the prevalence of pests and diseases (Ogbe, 2001). Viruses constitute a major threat to its production. African cassava mosaic disease (ACMD) and East African cassava mosai c disease (EACMD) caused by *cassava mosaic viruses*, family *Geminiviridae* is the most devastating in Nigeria. *Bemisia tabaci* Genn. is the vector of *Cassava mosaic virus* (ACMV) (Alegbejo, 2015) with B-biotype specific to cassava (Alegbejo and Banwo, 2006). In addition to cassava mosaic virus (CMV), Cassava Congo sequivirus also infect cassava and found in some farms where cassava is grown. It belongs to the family *sequiviridae* and vectored by *Aphis* spp (Uniprot, 2014). These viruses are also transmitted by infected stem cuttings. Yield losses with individual cassava cultivars range from 20 to 95% giving an average of 50 per cent (Alegbejo, 2015). There are lots of reports on cassava viruses in Southern Guinea, Rain forest and derived savannah zones (Akano, 1997), but little reports from Northern Guinea and Sudan savannah zones have been reported. Therefore, there is need to bridge the gap that exist. Hence, research was conducted in Kaduna (Northern guinea savannah zone) and Sokoto (Sudan savannah zone) to determine the incidence and distribution of cassava viruses for effective diagnosis and management of these viruses.

MATERIALS AND METHODS

Description of Study Areas

A survey was conducted in Kaduna and Sokoto States of Nigeria, September, 2015. The areas surveyed were Lere, Chikun and Kajuru Local Government Areas (LGAs) in Kaduna State (Figure 1) and Tureta, Shagari and Tambuwal LGAs, Sokoto State (Figure 2). Co-ordinates of the surveyed locations ranged from longitudes N10.24560 in Lere, Kaduna State to N12.63340 in Tureta, Sokoto State and latitudes E005.19010 in Tureta, Sokoto State to E008.10390 in Lere, Kaduna State. Highest altitude 678m (above sea level) in a Gurugu dam, Kajuru LGA, Kaduna State and the lowest was 228m (above sea level) in Mamia village, Tambuwal LGA, Sokoto State. .Fields were selected at random.

A total of 270 symptomatic and asymptomatic cassava leaf samples were collected from 18 farms and analysed for presence of virus by serological test (ELISA). In each field, 15 symptoms

and symptomless leaves samples were snapped collected and placed in polyethene bags and kept in cool box. Virus incidence was calculated according to (Chaube and Pundhir, 2006) as the number of symptomatic plants expressed as a percentage of the total number of plants assessed. Using GPS (Germin handheld), readings of altitude, latitude and longitude of each site were recorded

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Figure 1: Map of Kaduna State showing three Local Government Areas (LGAs)

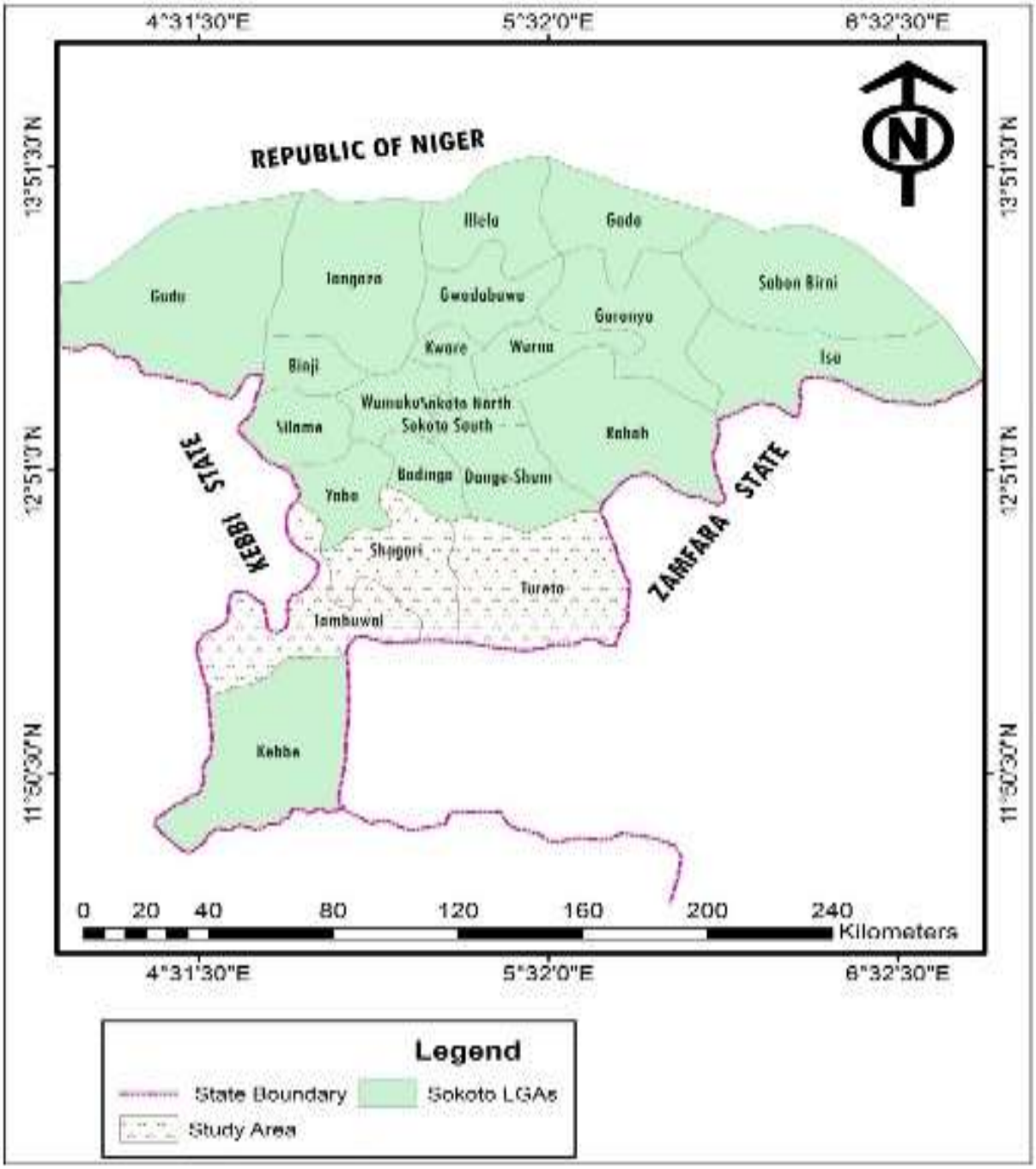

Figure 2: Map showing LGAs surveyed in Sokoto State

Triple Antibody and Double Antibody ELISAS

Triple antibody sandwich and Double Antibody sandwich ELISA were basically conducted as described by (Thomas *et al*., 1989) with minor modifications using polyclonal antisera (AS-0421/2) and (AS-041/4) raised against particles of ACMV and EACMV respectively for coating and monoclonal antibodies that are specific for each virus. Polyclonal antiserum (AS-0896) raised against particles of *Cassava Congo sequivirus* was used for coating the plate. Reference virus isolates were sourced from Duetsche Sammlung von Microorganismen und Zelikulturen (DSMZ) Braunschweig, Germany. ELISA plates were used to carry the experiments. Reactions were read after 1 hour at room temperature using Dynex MRx plate reader at absorbance A_{405nm}

Samples were considered positive when an average absorbance reading at 405nm is more than twice that of healthy (negative) controls (Kumar, 2009). Data were subjected to descriptive statistics (bar and pie charts) using Microsoft Excel version (12.0).

RESULTS

The ELISA test revealed the presence of three viruses; ACMV, EACMV, *Cassava Congo sequivirus* and mixed infections of ACMV +EACMV and ACMV + *Cassava Congo sequivirus* in the two States. ACMV was higher in Kaduna than Sokoto State while EACMV, *Cassava Congo sequivirus* and ACMV +EACMV were higher in Sokoto than Kaduna State as shown in Figure 5. In Kaduna State, ACMV was found to be positive in all the local government areas surveyed with highest incidence in Lere LG (68.89%) and the least was Kajuru LGA. Chikun LG had the highest incidence of EACMV (15.56%) while no incidence was observed in Kajuru. Kajuru LG was the only area with Cassava Congo virus incidence. Mixed infections of ACMV + EACMV and ACMV + Cassava Congo sequivirus were detected in Chikun LG while in Lere and Kajuru, mixed infections of ACMV + EACMV and ACMV + Cassava Congo sequivirus were found in each area respectively as shown in Figure 3. In Sokoto State, Tureta LG had the highest ACMV (43.3%) incidence while least was Tambuwal (11.0%). Tambuwal (22.23%) recorded highest EACMV incidence than Tureta and Shagari. The incidences of Cassava Congo sequivirus and co- infections of ACMV + EACMV and ACMV + Cassava Congo sequivirus were recorded in all LGAs as shown Figure in 4.

Symptoms of ACMV observed during the survey ranged from light green mosaic or yellow mosaic leaf colouration, leaf distortion, and shriveling (Plate 2), EACMV symptoms were dark green mosaic, distortion and stunting (Plate 4) and *Cassava Congo sequivirus* symptoms were localized chlorotic, mild mosaic with blisters and distorted leaves as in (Plate 3). Symptom expression was dependent on cassava cultivars and environmental conditions in which the crop was cultivated. However, some cultivar such as *Dan wari* and *Dan kurya* showed high virus incidence than *Bakin rogo* shown in (Tables 1 and 2).

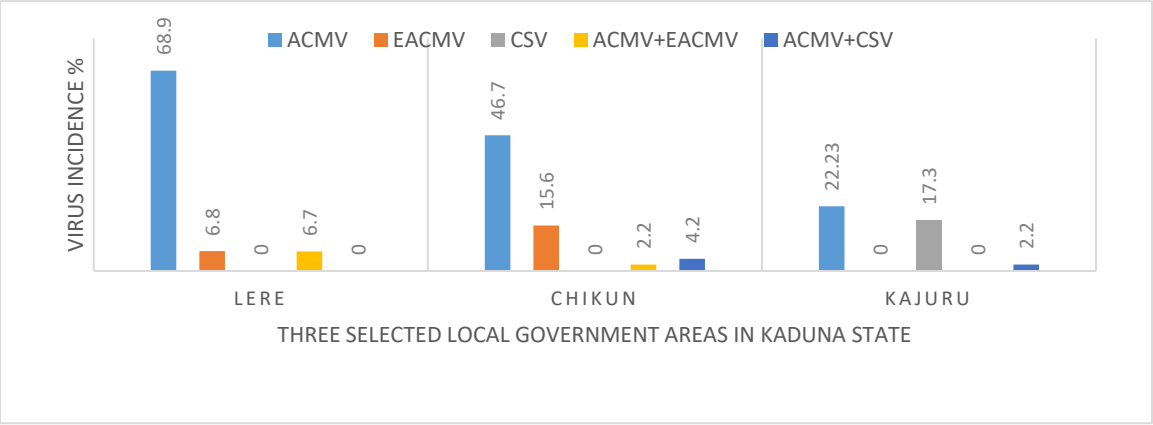

Figure 3: Virus incidence in three Local Government Areas in Kaduna State

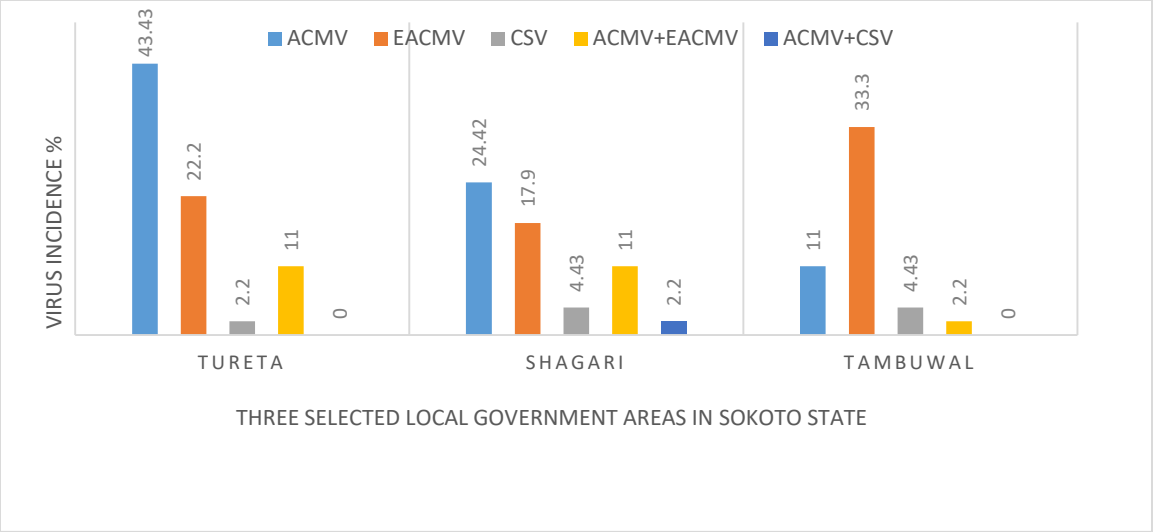

Figure 4: Virus incidence in three Local Government Areas in Sokoto State

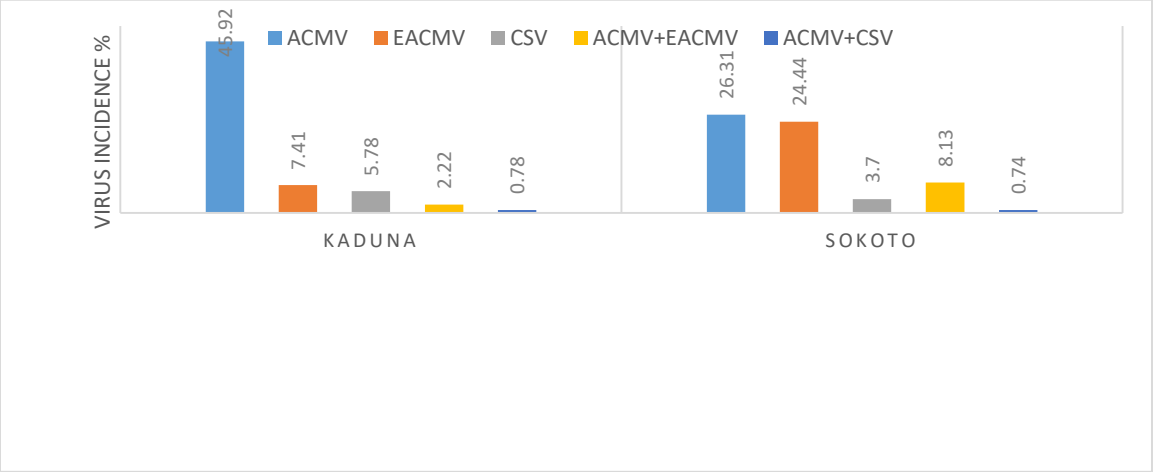

Figure 5: Comparison of virus incidences in Kaduna and Sokoto State

Distribution of cassava viruses

Based on the survey and laboratory results maps of the two States showing the distribution of the viruses were drawn (Figures 6 and 7).

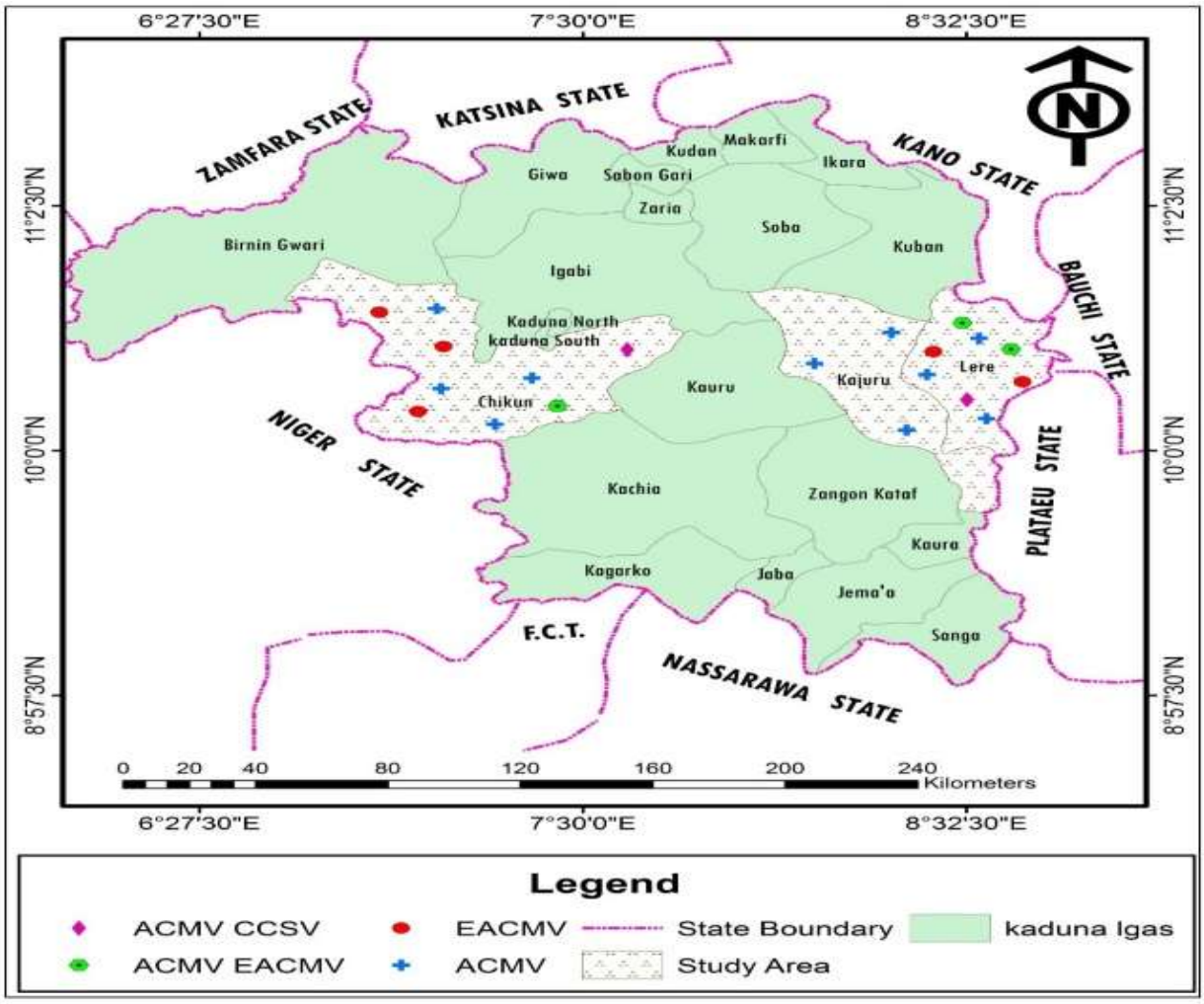

Figure 6: Distribution of viruses in Kaduna State

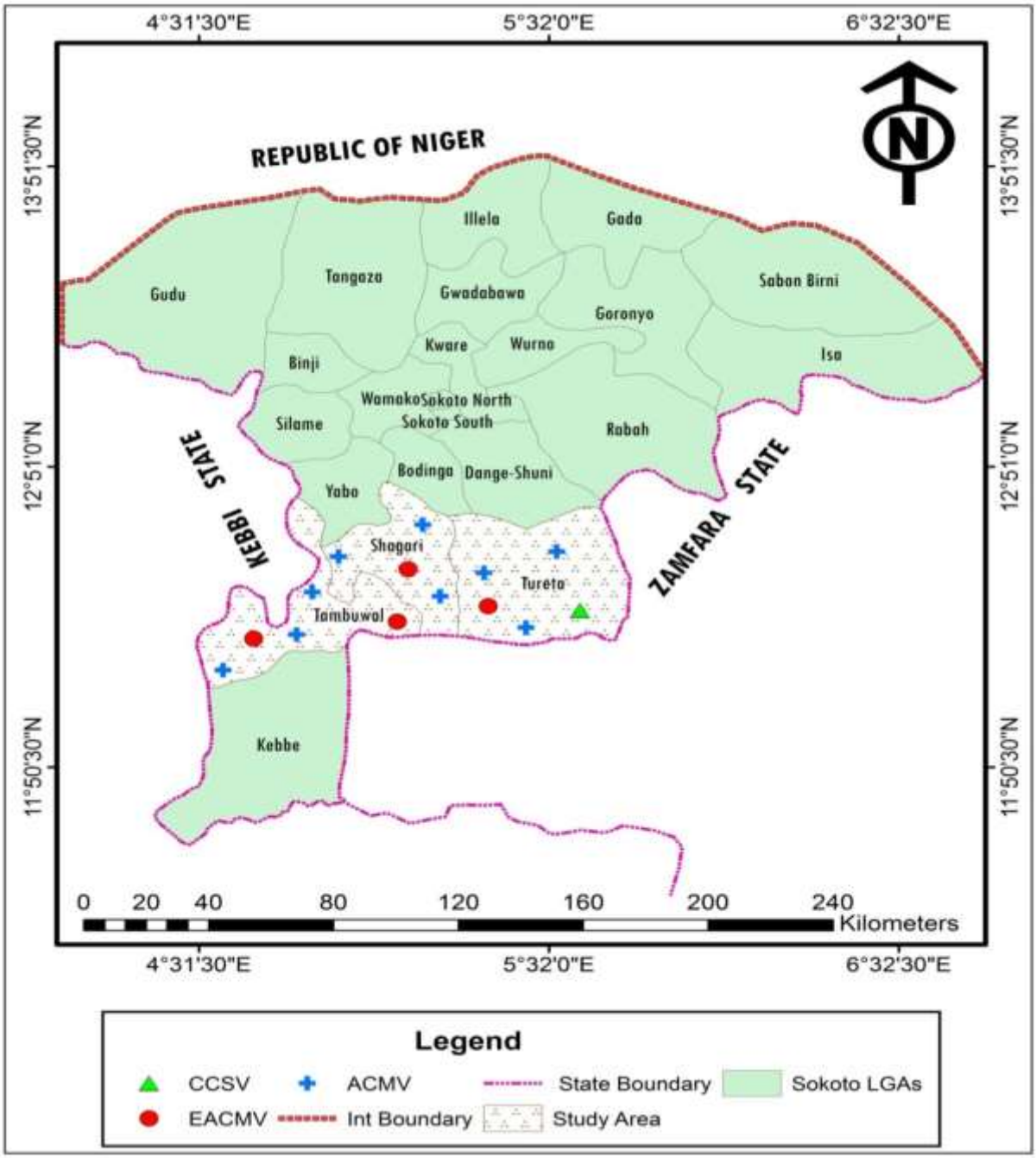

Figure 7: Distribution of viruses in Sokoto State

Plate 1: Healthy cassava

Plate 3: Cassava Congo Sequivirus symptom

Plate 4: EACMV symptoms

Table 1: Survey and cropping information of the locations surveyed in Kaduna State during the 2015 wet season.

LGA	Locations	Altitude (m)	Virus Incidence %					Cropping pattern	Crops surrounding the farm	Source of cassava cuttings	Cassava cultivars
Lere	Sigau	168	86.6	13.3	0	6.7		Mixed with okro and pepper	Cowpea and maize	P/H	D/wari
	A/ Kauche	183	73.3	6.7	0	6.7		Mixed with maize	Sorghum, maize, cassava	P/H	D/wari
	Dogon Daji	198	46.6	0	0			Mixed with cowpea, maize	Sorghum, maize,	P/H	D/kurya
Chikun	Dutsi	234	60.0	33.3	0	0	6.7	E/plant, pepper and tomato	Solaneceous crops	P/H	B/ rogo
	Chikuri	265	60.0	6.7	0	0	6.7	Mixed with beans	Tomato and pepper	P/H	D/wari
	A/madugu	280	20.0	6.7	0	0		Mixed with tomato	Tomato and pepper	P/H	B/ rogo
Kajuru	D/gayya	655	6.7	0	0	0		Sole cropping	Sorghum	P/H	D/wari and B/ rogo
	Gurugu dam	658	6.7	0	13.3	0	6.7	Sole cropping	Sorghum	P/H	D/wari
	Kujama	661	53.3	0	40.0	0		Sole cropping	Mixed with cowpea	P/H	D/wari

%= percentage, ha= hectare, A= Anguwan, Dutsen, D/wari= Dan wari, Dan kurya, B/rogo, Eggplant, P/H= Previous Harvest and NIL= Not at all

Table 2: Survey and cropping information of the locations surveyed in Sokoto State during the 2015 wet season.

LGA	Locations	Altitude (m)	Virus Incidence %					Cropping pattern	Crops surrounding the farm	Source of cassava cuttings	Cassava
Tureta	L/Tureta	178	50.3	26.6	6.7	13.3	0	Mixed with okro, tomato, maize	Millet and moringa	Marafa market	D/wari
	Tureta	261	40.0	26.6	6.7	13.3	0	Mono cropping	Sorghum, millet, cassava	Marafa market	D/wari
	G/yausi	263	40.0	13.3	0	6.7	0	Mono cropping	Sorghum, millet,	Tureta market	D/ kurya
Shagari	K/mallam	253	33.3	26.6	13.3	13.3	6.7	Intercropping	Millet	P/H	D/sagam
	Kanbama	248	26.6	13.3	0	13.3	0	Mixed with beans	Millet	P/H	Sagaf
	Mazoji	246	13.3	13.3	0	6.7	0	Mixed with millet	Millet	P/H	Sagam
Tambuwal	Larbardo	243	26.6	46.6	6.7	6.7	0	Sole cropping	Maize and cowpea	P/H	D/wari and B/rogo
	Maniya	232	6.7	33.3	0	0	0	Sole cropping	Sorghum	P/H	D/wari
	Maima	228	0	20.0	0	0	0	Intercropping with solanceous	Eggplant, maize and sorghum	P/H	D/wari

%= percentage, ha= hectare, L/Tureta= Lambar Tureta, G/yausi= Gidan yausi, K/mallam = Kafin mallam, D/wari= Dan wari, Dan kurya, B/rogo, P/H= Previous Harvest and NIL= Not at all

DISCUSSION

The serological test conducted accurately affirmed that the causal agents of the aforementioned symptoms were the viruses. The symptoms of AMCV and EACMV observed on the samples included leaf curl, mosaic, mottling, distortion, stunting, chlorosis, and necrosis. Fondong *et al.,* (2000) and Ogbe *et al*., (2003) reported earlier that CMD symptoms observed depends on virus species, strain and mixed infections. These symptoms observed on the samples for ACMV and EACMV were the same as what was reported by (Ogbe *et al.,* 2006) but *Cassava Congo sequivirus* showed mild chlorotic with blisters on leaf surface and leaf distortion.. The results indicate ACMV as the most predominant virus detected, followed by EACMV and least was *Cassava Congo Sequivirus*. This confirmed the work of (Sseruwagi *et al.,* 2004) that ACMV has been known to occur in most of cassava producing areas of Africa. There are several reports indicating the prevalence of ACMV in African countries and the most important virus disease of cassava in Togo (Adjata *et al.,* 2008 and Dansou- kodja *et al*., 2017), Uganda (Harrison *et al*., 1997), Kenya (Karakacha, 2001), and Nigeria (Ogbe *et al*., 2003)

In this study, ACMV and EACMV have wider range of altitude, ranging from 168 to 661m (above sea level) than cassava Congo sequivirus. Incidence of cassava viruses was influenced by environmental conditions at the time of infection. High virus incidences were found in lower altitude areas. This was in agreement with the report of (Osogo *et al.,* 2014) that high leaf incidence occurs in low altitudes areas while low leaf incidence occurs in high altitudes. Secondly, the use of local cutting stem

such as *Dan wari*, *Dan kurya*, *Sagam* and *Bakin rogo* which were cultivated year in year out also contribute to high virus incidence as shown in (Tables 1 and 2). Earlier report by Samura *et al.,* (2014) that the wide spread of ACMV can be attributed to widely use of local varieties which are suscepticible to disease and Nzue *et al.,* (2005) also reported CMD perpetuate through infected cuttings.

Another reason for high virus incidence was the cropping pattern where cassava crop was intercropped and also surrounded with solanaceous crops such as tomato pepper and dicotyledonous such as cowpea. However, low virus incidence was observed when intercropped or surrounded by monocotyledonous crops such as maize, millet and sorghum. This observation was in conformity with the finding of (Thresh and Cooter, 2005) that solaneceous plants were attributed to high disease incidence in the farms surveyed.

Cassava Congo sequivirus was found in single and mixed infections in high altitudes probably because susceptible cultivars were grown, both in single crop cultivation and mixed cropping with solaneceous crop. Mixed infections were found in some farms, and Alabi *et al.,* (2008) showed the possibilities of co- infection in Africa.

A comparison between Kaduna and Sokoto States showed that ACMV had higher occurrence in Kaduna than Sokoto State with marked differences. The current status of the disease in these two States may be due to increase in production of cassava in Kaduna than Sokoto. The spread of EACMV was higher in Sokoto than Kaduna. (Ogbe *et al.,* 2006) reported that the virus spread widely but with low occurrence in some parts of Nigeria as was reported. *Cassava Congo Sequivirus* which belongs to the family *Sequiviridae* was not widely distributed in Kaduna and Sokoto States of Nigeria. This the first report of this virus to the best of my knowledge. (Yepes *et al.,* 2014) detected the presence of new virus that belong to family *Secoviridae* in Brazil.

CONCLUSION

Cassava viruses were present in all surveyed areas with an even spread in Sokoto State. *African Cassava mosaic virus* (ACMV) had the highest incidence and most widely distributed virus in this study. *East African cassava mosaic virus* (EACMV) had a wide spread but low in occurrence while *Cassava Congo Sequivirus* was not widely spread and had low occurrence. Therefore, understanding the distribution of single and mixed infections could be used in preventing further spread of these viruses. This is the first report of *Cassava Congo Sequivirus* in Kaduna and Sokoto States, Nigeria.

RECOMMENDATIONS

The use of susceptible cutting stems should be discouraged so as to avoid the spread of viral disease incidences. Farmers should be mindful on the crops they intercropped with cassava to minimize virus occurrence because solaneceous crops such as tomato, eggplant and pepper, and dicotyledonous crops such as cowpea and soybeans attribute to high incidence.
Molecular characterization of *Cassava Congo sequivirus* should be done to know the relationship with other viruses.

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