Science World Journal Vol. 15(No 2) 2020
www.scienceworldjournal.org
ISSN 1597-6343
Published by Faculty of Science, Kaduna State University

Full Length Research Article

ALTERNATIVE HOSTS OF CASSAVA VIRUSES IN KADUNA AND SOKOTO STATES, NIGERIA

H. Badamasi[1*], M. D. Alegbejo[2], B. D. Kashina[2] and O. O. Banwo[2]

[1]Pest Management Programme, Samaru College of Agriculture, Division of Agricultural Colleges, Ahmadu Bello University, Zaria
[2]Crop Protection Department, Faculty of Agriculture, Ahmadu Bello University, Zaria

Corresponding Author's Email Address: hbadamasi22@gmail.com Phone: +23481758383230

ABSTRACT
Field surveys were conducted in 2015 wet and 2016 dry seasons to determine the occurrence of alternative hosts of cassava viruses in Kaduna and Sokoto States, Nigeria. Eighteen farms from six local Government Areas namely; Lere, Chikun, Kajuru (Kaduna State), Tureta, Shagari and Tambuwal (Sokoto State) were surveyed. Fifty- four weed samples within and around the farms were collected; Eighteen weeds were identified in wet season while 19 weeds were collected and 18 were identified during dry season. Three viruses were tested; *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV) were detected using Triple Antibody Sandwich ELISA and *Cassava Congo sequivirus* using Double Antibody Sandwich ELISA. In Kaduna State, seven samples were positive to ACMV (38.8%) and four samples were positive to ACMV (22.22%) in wet and dry seasons respectively. One sample was positive to EACMV (5.56%) and mixed infection of ACMV + EACMV (5.56%). *Cassava Congo sequivirus* was negative in all the samples. In Sokoto State, seven weeds were positive to ACMV (38.89%) and three weeds were positive to ACMV (16.69%) in both wet and dry seasons respectively. Weeds that were identified in both wet and dry seasons were *Combretum hispidum* (L.) and *Euphorbia hirta. Euphorbia hirta* (L) was found to be an alternative host to ACMV, EACMV and their co-infection. The identification of *Euphorbia hirta* as new alternative host has widen the knowledge on viral inoculum. This will help to narrow the gap in spread of the disease.

Keywords: Cassava, Viruses, Alternative Hosts and Occurrence

INTRODUCTION

Cassava viruses: *African cassava mosaic virus* and *East African cassava mosaic virus* are transmitted by whitefly *Bemisia tabaci* and propagation of infected stem cuttings (Geddes, 1990; Legg *et al.,* 1992 and Thresh *et al.,* 1994). In addition, alternative hosts serve as source of inocula for new infections to susceptible cultivars even if cassava virus-free planting materials were used (Hillocks, 2003). They play important roles in the ecology and epidemiology of viruses (Kazinczi *et al*., 2007). Wild cassava plant *Manihot glaziovii* Mull was identified as the only alternative host of Cassava mosaic virus CaMV in Mozambique (Hillocks, 2003). No alarm was raised because the species is a close relative of cassava. With the increase of alternative hosts including the non-cassava host plants became great concern among epidemiologist and the disease management experts. This is probably due to wide host range of whitefly which is the vector of CaMV (Mware *et al.,* 2010). *B. tabaci* being the most important, transmitting about 111 virus species (Brown *et al*., 1995) including cassava mosaic Gemini-viruses (CMGs) (Legg *et al.,* 1992) and they transmit at least 21 viruses in Nigeria (Alegbejo, 2000). In Nigeria, natural host and weed hosts of CaMV have been reported with occurrence of Cassava mosaic disease (CMD) on originally healthy cassava crop associated with weed hosts has been reported (Hillocks, 2003). No weed host(s) for *Cassava Congo Sequivirus* was reported. However, Secundina plant was used as an experimental host for members of family *Sequiviridae* (Wilmer *et al*., 2015). Therefore, the needs to detect the presence of ACMV, EACMV and *Cassava Congo sequivirus* on non-cassava plants is necessary in order to fill the gap that exist.

MATERIALS AND METHODS

Surveys for Alternative Host of Cassava Viruses.

Alternative hosts were surveyed in 2015 wet and 2016 dry seasons in Lere, Chikun, Kajuru Local Government Areas (Kaduna State) and Tureta, Shagari and Tambuwal Local Government Areas (Sokoto State). Both broad and narrow leaves species within and around the farms were randomly collected and labeled. The weed plants were identified to species level in the Herbarium (Plant Collection) Botany Unit, Faculty of Science, A.B.U. Zaria, Samples collected were stored in the refrigerator at 4˚C for later use.

Samples collected

Fifty- four weed samples were collected from the farms in wet season and only18 weed samples were identified. Nineteen weeds were collected in dry season and 18 weeds were also identified.

Detection of Viruses in Weed Hosts Using Triple Antibody Sandwich and Double Antibody Sandwich ELISA.

Triple antibody sandwich and Double Antibody sandwich source of Elisa kits 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 Zelikultuern (DSMZ), Braunschweig, Germany.

ELISA plates were coated with two hundred microlitre of polyclonal antibody diluted at 1:1000 in carbonate buffer. The plates were covered and incubated at 37 °C for 2 h and washed by flooding three times with PBS-Tween 20 (PBS-T), drained and tapped dry. Two hundred microlitre of 2 % skimmed milk in PBS

Tween was added into each well to block the uncoated sites. The plates were covered and incubated at 37 °C for 30 min. The blocking solution was then removed and tapped dry. Weed samples were extracted in extraction buffer by grinding samples in extraction buffer (1: 20 w/v). Two hundred microlitre aliquots of the test samples were added to duplicate wells. The plates were covered and incubated at 37 °C for 2 h, then the plates were washed three times with PBS-T by flooding three times, drained and tapped dry. Two hundred microlitre of the monoclonal antibody (MAb) diluted in conjugate buffer was then added to each test well. The plates were covered, incubated at 37 °C for 2 h and washed as described earlier. For TAS ELISA an additional antibody rabbit anti-mouse alkaline phosphatase (RAM-AP) was diluted at 1:1000 in conjugate buffer and added into the plates. The plates were covered and incubated at 37 °C for 2 h and washed times with PBS-Tween by flooding washed times, drained and tapped dry. Two hundred microlitre of p-nitrophenyl phosphate substrate was added per well and incubated at room temperature in the dark for 1 h. The plates were read after 1 h using spectrophotometric measurement of absorbance at A405 nm (Clark and Adams, 1977). ELISA values at least twice that of the negative control (check) were rated positive according to Kumar (2009).

Data Analysis

Data obtained were subjected to inferential statistical analysis: percentages and pie charts.

RESULTS

Incidence of ACMV, EACMV and *Cassava Congo sequivirus* on Alternative Hosts

Seven weed samples out of eighteen (38.89%) were infected by ACMV. One out of eighteen (5.56%) was infected by EACMV. *Cassava Congo sequivirus* was not detected and only mixed infection of ACMV+ EACMV was detected in one out of eighteen weed samples in Kaduna State (Figure 1). ACMV was detected in four out of eighteen weed sample (22.22%). EACMV was detected in one out of eighteen (5.55%) and *Cassava Congo sequivirus* was not detected in any of the samples (Figure 1). In Sokoto State, ACMV was the only virus detected. Seven out of eighteen (38.89%) tested positive in wet season and three weed samples detected positive to ACMV (16.67%) (Figure 2).

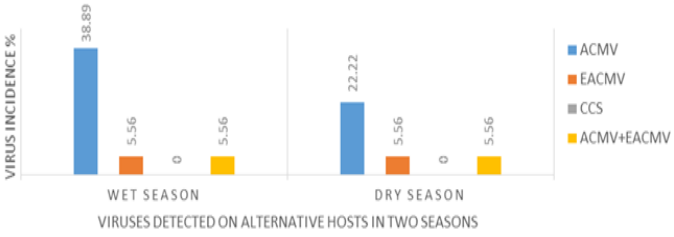

Figure 1: Incidence of cassava viruses on alternative hosts for two seasons in Kaduna State

Key:
ACMV, African cassava mosaic virus
EACMV, East African cassava mosaic virus
CCS, Cassava Congo sequivirus

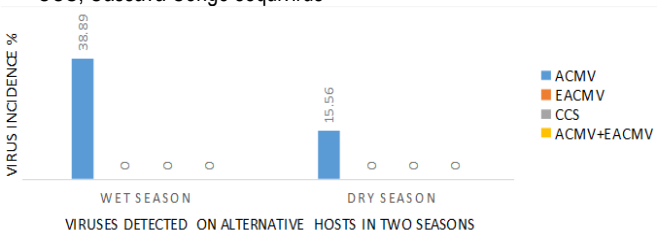

Figure 2: Incidence of cassava viruses on alternative hosts for two seasons in Sokoto

Key:
ACMV, African cassava mosaic virus
EACMV, East African cassava mosaic virus
CCS, Cassava Congo sequivirus

Occurrence of ACMV, EACMV and *Cassava Congo sequivirus* on Alternative Hosts

In the 2015 wet season, *Combretum hispidum* L., *Aneilima beniniense* L., *Mitracarpus villosus* L., *Ageratum conyzoides* L., *Ficus exasperate* L., *Euphorbia hirta* L., *Laportea aestuans* L.were detected positive to ACMV antigen while *Euphorbia hirta* L and *Ageratum conyzoides* L were positive to EACMV antigen. *Cassava Congo sequivirus* and mixed infections were not observed in any weeds in Kaduna State (Table1). For the 2016 dry season, the four weeds identified were *Laportea aestuans* L., *Combretum hispidum* L., *Ageratum conyzoides* L. and *Euphorbia hirta* L. Euphorbia hirta L was the only weed host positive to EACMV antigen and no any weed hosts were found positive to *Cassava Congo sequivirus* antigen and mixed infections of the viruses.

In Sokoto State, ACMV antigen was detected in the following weeds: *Aneilima beniniense* L., *Mitracarpus villosus* L., *Ageratum conyzoides* L., *Ficus exasperate* L., *Euphorbia hirta* L., *Solanum nigrum* L. EACMV antigen was detected in *Ageratum conyzoides* L. while *Cassava Congo sequivirus* and mixed infections were not observed in any of the weeds tested (Table 2). In the dry season, ACMV antigen was detected in *Laportea aestuans* L., *Ageratum conyzoides* L. and *Euphorbia hirta* L. only (Table 2).

Science World Journal Vol. 15(No 2) 2020
www.scienceworldjournal.org
ISSN 1597-6343
Published by Faculty of Science, Kaduna State University

Table 1: Distribution of ACMV, EACMV and *Cassava Congo sequivirus* on Alternative Hosts in Kaduna State

		Wet season 2015			Dry season 2016			**Mixed Infection**			
Families of weeds tested	Scientific names of weeds	ACMV	EACMV	*Cassava Congo sequivirus*	ACMV	EACMV	*Cassava Congo sequivirus*	ACMV/EACMV	ACMV/ *Cassava Congo sequivirus*	EACMV/ *Cassava Congo sequivirus*	ACMV/EACMV/ *Cassava Congo sequivirus*
Poaceae	*Pennisetum pedicellatum*	-	-	-	-	-	-	-	-	-	-
	Sorghum arundinaceum	-	-	-	-	-	-	-	-	-	-
	Paspalum scrobiculatum	-	-	-	-	-	-	-	-	-	-
Commelinaceae	*Aneilima beniniense*	+									
	Spreading day flower	-	-	-	-	-	-	-	-	-	-
Rubiaceae	*Mitracarpus villosus*	+	-	-		-	-	-	-	-	-
Cyperaceae	*Kyllinga squamulata*	-									
Asterceae	*Ageratum conyzoides*	+	+	-	+	-	-	-	-	-	-
Moraceae	*Ficus exasperate*	+									
Acanthaceae	*Monechma ciliatum*	-	-	-	-	-	-	-	-	-	-
Loganiaceae	*Spigelia anthelmia*	-									
Convolvulaceae	*Morning glory weed*	-	-	-	-	-	-	-	-	-	-
Euphorbiaceae	*Euphorbia hirta* Linn	+	+	-	+	+	-	+	-	-	-
Solanaceae	*Solanum nigrum*	-	-	-	-	-	-	-	-	-	-
Urticaceae	*Laportea aestuans*	+			+						
Combretaceae	*Combretum hispidum*	+	-	-	+	-	-	-	-	-	-
Tiliaceae	*Triumfetta rhomboidea*	-			-						
Amaranthaceae	*Amaranthus spinosus*	-	-	-	-	-	-	-	-	-	-

ACMV= *African cassava mosaic virus* Red, EACMV = *East African cassava mosaic virus* Green, + = detection, - = no detection.

Table 2: Distribution of ACMV, EACMV and *Cassava Congo sequivirus* on Alternative Hosts in Sokoto State

		Wet season 2015			Dry season 2016			Mixed Infections			
Families of weeds tested	Scientific names of weeds	ACMV	EACMV	*Cassava Congo sequivirus*	ACMV	EACMV	*Cassava Congo sequivirus*	ACMV+ EACMV	ACMV+ *Cassava Congo sequivirus*	EACMV+ *Cassava Congo sequivirus*	ACMV+EACMV+ *Cassava Congo sequivirus*
Poaceae	*Pennisetum pedicellatum*	-	-	-	-	-	-	-	-	-	-
	Sorghum arundinaceum	-	-	-	-	-	-	-	-	-	-
	Paspalum scrobiculatum	-	-	-	-	-	-	-	-	-	-
Commelinaceae	*Aneilima beniniense*	+									
	Spreading day flower	-	-	-	-	-	-	-	-	-	-
Rubiaceae	*Mitracarpus villosus*	+	-	-		-	-	-	-	-	-
Cyperaceae	*Kyllinga squamulata*	-									
Asterceae	*Ageratum conyzoides*	+	+	-	+	-	-	-	-	-	-
Moraceae	*Ficus exasperate*	+									
Acanthaceae	*Monechma ciliatum*	-	-	-	-	-	-	-	-	-	-
Loganiaceae	*Spigelia anthelmia*	-									
Convolvulaceae	*Morning glory weed*	-	-	-	-	-	-	-	-	-	-
Euphorbiaceae	*Euphorbia hirta* Linn	+	+		-	-	-	-	-	-	-
Solanaceae	*Solanum nigrum*	-	-	-	-	-	-	-	-	-	-
Urticaceae	*Laportea aestuans*	+			+						
Combretaceae	*Combretum hispidum*	+	-	-	-	-	-	-	-	-	-
Tiliaceae	*Triumfetta rhomboidea*	-			-						
Amaranthaceae	*Amaranthus spinosus*	-	-	-	-	-	-	-	-	-	-

ACMV= *African cassava mosaic virus* Red, EACMV = *East African cassava mosaic virus* Green, + = detection, - = no detection

DISCUSSION

This study revealed two newly identified weed hosts; *Combretum hispidum* L.*,* and *Euphorbia hirta* L. Earlier, Shoyinka *et al.* (2001) reported that castor oil plant (*Ricinus communis* L.) as a natural host of ACMV and EACMV in Nigeria. In a similar study, Ogbe (2001) reported *Senna occidentalis* (L.) as new natural host of ACMV. Briddon *et al*. (2008) also reported *Ageratum conyzoides* L. and *Laportea aestuans* L. as alternative weed hosts of ACMV in Southern Africa. Mixed-infections of ACMV +EACMV were detected in *Euphorbia hirta* L in this study. This is line with several reports that there exist mixed infection of ACMV+ EACMV in *Senna occidentalis* (L.) and *Combretum confertum* (L.) Ogbe, (2001) and Alabi *et al.* (2008) also reported that *Senna occidentalis, Leucana leucocephala, Glycine max, Ricinus communis, Combretum confertum* and *Manihot glaziovii*. Ramkat *et al.* (2011) also reported co-infection of ACMV and *East African cassava mosaic virus*- Ugandan variant (EACMV-UG) on *Jatropha carcus* in Kenya. This shows the existence of more than one virus either singly or in mixed culture in a sample. Alternative hosts play an important role in virus disease epidemiology. Studies by Kashina *et al.* (2002) and Alegbejo (2015) revealed that most weeds and other cultivated crops around served as reservoir hosts to plant viruses. Therefore, weed hosts identified in this study could act as source of inoculum to cassava viruses that were identified. The perennial nature of *C. hispidum* and *E. hirta* that were widely found on the farms could provide a constant reservoir for effective transmission by vectors. Similar findings were reported by Alabi *et al*. (2008) that *Senna occidentalis* and *C. confertum* widely present in cassava-growing regions would provide a reservoir for cassava mosaic begomoviruses (CMBs) year round. Ibrahim *et al*. (2017) reported that *Euphorbia hirta* L was not only a reservoir host of ACMV, EACMV and co- infection but to other important plant viruses. *Cassava Congo sequivirus* and other mixed infections were not detected in any of the weed. The newly identified natural hosts of ACMV and EACMV in this study could help limit the epidemiological gap that exist in predicting the occurrence and spread of the viruses.

Conclusion

The newly identified natural hosts of ACMV namely: *C. hispidum* and *E. hirta* and EACMV and mixed infection ACMV+ EACMV identified on *E. hirta* in this study could go a long way in linking the epidemiological knowledge gap that play vital role in virus transmission. However, no any weed host of *Cassava Congo sequivirus* was found.

Recommendations

Farmers should employ regular weeding mechanisms to avoid weeds build up that harbor cassava viruses in both dry and wet seasons. Further studies should be conducted to identify more alternative hosts of cassava viruses in other cassava growing areas so as to link the gap that exist in epidemiological knowledge.

Acknowledgement

I am sincerely grateful to Samaru College of Agriculture, Division of Agricultural Colleges, Ahmadu Bello University, Zaria for financing the research. Also, I appreciate the technical services rendered by Messrs: A. Malik and J. Sedi, and Mrs H. Wakawa during the research.

REFERENCES

Alegbejo, M. D. (2015). Virus and virus-like diseases of crops in Nigeria. Zaria Ahmadu Bello University Press. 202-203pp.

Alegbejo, M. D. 2000. Evaluation of okro cultivars for resistance to leaf curl virus in okro germplasm. *Journal of Agricultural Technology,* 8 (1): 1-4.

Briddon, R. W., Baguma, Y. K., Ssemakula, G. N., Bull, S. E., Bua, A., Alicai, T., Omongo, C., Otim-Nape, G. W., Sserubombwe, W. S. and Stanley, J. Diversity of begomoviruses associated with mosaic disease of cultivated cassava (*Manihot esculenta* Crantz) and its wild relative (*Manihot glaziovii* Mull. Arg.) in Uganda. *Journal of General Virology,* 9: 1759 - 1769.

Brown, J. K, Frohlich D. R, Rosell R. C. (1995). The sweet potato silver leaf whiteflies: Biotypes of *B. tabaci,* a species complex. *Annual Review of Entomology,* 40:511-534.

Clark, M. F. and Adams. A. N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology,* 4: 475-483

Geddes A. M. W. (1990). The relative importance of crop pests in sub-Saharan Africa. NRI Bulletin No. 36, Chatham, United Kingdom.

Hillocks, R. J. (2003). Cassava brown streak virus disease: past, present and future. Proceedings of an International Workshop, Mombasa, Kenya, October 2003. pp. 30 – 100.

Ibrahim, B., Alegbejo, M. D., Kashina, B. D. and Banwo, O. O. (2017). Incidence, distribution and alternative hosts of viruses infecting tomato in Sokoto and Zamfara States, Nigeria. Unpublished thesis, Ahmadu Bello University, Zaria Pp 56.

Kashina, B. D., Mabagala, R. B. and Mpunami, A. A. (2002a). Reservoir weed hosts of Tomato yellow leaf curlbegomovirus from Tanzania. *Archives of Phytopathology and Plant Protection, 3*5 (4): 269–278.

Kazinczi, G., Horvath, J. and Takacs, A. (2007). Toposviruses on ornamental plants. *Plant Viruses, 2*: 142- 162.

Kumar, P. V. (2009). Methods for the Diagnosis of Plant Virus Diseases. Laboratory Manual. Virology and Molecular Diagnosis Unit, IITA-Ibadan. Pp 33-38.

Legg, .P, Asiimwe P., Sseruwagi P., Brown J. K. (1992). Changes in the biology of *Bemisia tabaci* on cassava in Africa and their impact on virus disease pandemics. Fourth International *Bemisia* Workshop: International Whitefly Genomics Workshop. Journal of Insect Science (8):4. http://www.insectscience.org/8.04/ref/abstract51.html

Mware, B. O. S., Ateka, E. M., Songa, J. M., Narla, J., Amata, R. D., Olubayo, F. and Amata, R. (2010). Transmission and distribution of cassava brown streak virus disease in cassava growing areas of Kenya. *Journal of Applied Bioscience,* 16: 864 – 870.

Ogbe, F. O., Atiri, G. I., Dixon, A. G. O. and Thottappilly, G. (2003). Serological and biological variations of *African cassava mosaic virus* in Nigeria. *Annals of Applied Biology*, 43: 203 - 213.

Ramkat, R. C, Calari, A., Maghuly, F. and Laimer, M. (2011). Biotechnological approaches to determine the impact of viruses in the energy crop plant *Jatropha curcas*. *Journal of virology,* 8:386.

Shoyinka, S. A., Thottappilly, G., McGrath, F.F. and Harrison, B. D. (2001). Detection, relationships and properties of

cassava mosaic geminivirus in naturally infected castor oil plant, *Ricinus communis* L. in Nigeria. Page 20 in: The Book of Abstracts, Fifth Conference of Cassava Biotechology.

Thomas, J. E., Massaloki, P. R. and Harrison, B. D. (1986). Production of monoclonal antibodies to African cassava mosaic virus and differences in their reactivity's with other whitefly transmitted geminiviruses. *Journal of General Virology*, 67: 2739 – 2748.

Thresh, J. M., D. Fargette, and G.W. Otim-Nape. (1994). Effects of African cassava mosaic Geminivirus on cassava yield. *Tropical Science* 34: 26–42.

Wilmer. J., Ivan. L., Maritza. C., Cristiano.O. Carvayal. M. and Castano. M. (2015). Cassava viruses: Characterisation and study of their interaction in plant affected by frog skin disease. *Virus Research,* 86: 76 - 86