MOLECULAR DETECTION OF AFRICAN CASSAVA MOSAIC VIRUS (ACMV) AND EAST AFRICAN CASSAVA MOSAIC VIRUS (EACMV) IN NORTHERN NIGERIA

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SUMMARY

Cassava mosaic Geminiviruses are among the most important biotic agents that cause Cassava mosaic disease, limiting the production potential of the crop in Nigeria. This study was conducted to evaluate the current status of Cassava mosaic disease incidence and also to detect the viruses (ACMV and EACMV) causing the disease in the six (6) States in North Eastern Nigeria (Bauchi, Yobe, Gombe, Borno, Adamawa and Taraba), between January and September, 2017. A total of forty-nine (49) farmers' fields were surveyed and, in each field, thirty plants were assessed regarding the CMD incidence. Leaf samples were collected from each field and prepared in the form of herbarium and transported to molecular biology laboratory, Kebbi State University of Science and Technololgy, Aliero, for virus diagnosis. The results of the study revealed that Cassava mosaic disease incidence varied among the states. It was highest in Gombe (73.90%) followed by Bauchi (63.70%) and lowest in Yobe (21.11%) State. Differential polymerase chain reaction (PCR) results using specific primers for African Cassava mosaic virus (ACMV) and East African Cassava mosaic virus (EACMV) revealed that 52.60% of the tested samples were positive to ACMV single infections and 14.10% were positive to EACMV single infections respectively. Dual-infections of ACMV and EACMV were detected in 10.40% of the tested samples. The use of resistant varieties as a means of managing the disease is recommended in the study area.

Key words: Disease, Cassava, Incidence, EACMV, ACMV

CASSAVA (*Manihot esculentus,* Crantz), belonging to Euphorbiaceae family, is a perennial shrub with edible roots which grows in tropical and subtropical areas of the world (23, 35). It originated from tropical America (Brazil) and was first introduced into Africa in Congo basin by Portuguese traders around 16th century (17). Cassava is the third most important source of carbohydrate in Sub-Saharan Africa (SSA) and an important food crop in Nigeria. (17). On a worldwide basis, the crop is ranked sixth most important source of calories in human diet (12, 16). Cassava generates cash income for a large number of households in comparison with other food staples (24, 25), making it an essential contributor to food security, poverty alleviation and economic growth in the SSA region (21). It is rich in carbohydrates, Calcium, Vitamins B and C and essential minerals (20, 28). It provides food security during conflicts when the invaders cannot easily destroy or remove the crop, since it conveniently grows underground (30).

Virus diseases cause serious problem to cassava production as they are transferred from one cropping cycle to next through stem cuttings that are used as planting material (29, 36). Researches (9, 13) have revealed that Cassava mosaic disease is caused by several distinct whitefly transmitted viruses [African cassava mosaic virus (ACMV), 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) and South African cassava mosaic virus (SACMV)].

These viruses belong to four genera: *Nepovirus*, *Begomovirus, Ipomovirus,and Caulimovirusas* as reported from cassava, (20, 26). Among these classifications, only *Ipomovirus*: *Cassava brown streak virus* (CBSV), *Cassava brown streak virus* Ugandan and *Begomovirus*: Cassava mosaic geminiviruses (CMGs) of the family *Geminiviridae* are of economic importance in Africa (25). Although CBSV is important in East Africa, CMGs are the most economically important viruses of Cassava in Africa (20, 33).

These viruses show different infection dynamics in terms of symptom expression, progression, recovery, severity, as well as host range (10, 31). The genome of each of the viruses consists of two subgenomic DNA components, DNA-A and DNA-B. DNA-A and DNA-B, each of about 2.8 kbp (30),

with different roles in the infection process. DNA-A encodes genes responsible for viral replication [AC1 (Rep), and AC3 (Ren)], regulation of gene expression [AC2 (Trap)] and particle encapsidation [AV1 (CP)] while DNA-B encodes two proteins, BC1 (MP) and BV1 (NSP) involved in cell-to-cell movement within the plant, host range and symptom modulation (29).

Cassava mosaic disease (CMD) in Africa was first described in the late 19th century and is now found wherever cassava is grown in the continent and is the single major constraint to Cassava production in West-Africa. It was reported to be spread by whitefly and the planting of infected cuttings (27, 32). Causal agents include the African Cassava mosaic virus (ACMV), East African Cassava mosaic virus (EACMV) found throughout the Cassava growing regions of Africa and East African Cassava mosaic virus-Ugandan (EACMV-Ug), which occurs alone in coastal East Africa and has been associated with severe Cassava mosaic disease pandemic in East and Central Africa. Cassava mosaic disease (CMD) is the most important cassava disease in Nigeria as it causes severe reduction on Cassava yield, up to 70%. (20). Studies by (26) Zambia have shown that Cassava mosaic disease incidence, spread, severity and the extent of yield loss depend on the variety susceptibility and stage of plant growth at which infection occurs. Recently, it was established that the severity of CMD is influenced by synergistic effects of co-infection of CMBs and its associated DNA satellites (26). Losses are attributed to damage on leaves and stems, which interfere with the way in which the plant makes food for storage in the roots. The damaged photosynthesis areas reduce the growth of the plants, number of storage roots and the ability of the storage roots to enlarge and mature. Loss of planting material also occurs in infected Cassava, where stem cuttings are unhealthy and unsuitable for planting (21).

This study was carried out to detect the virus strains that are causing cassava mosaic disease (CMD) in North eastern Nigeria. Provision of these information would be of great benefit to cassava growers, researchers especially breeders and virologists, as well as policy makers. This will also help in identifying disease hot spot areas for deployment of clean planting materials to farmers. The information will also help in guiding decisions in choosing areas suitable for cassava seed multiplication.

MATERIALS AND METHODS

Study Area.

North east Nigeria is characterized by Sahel climatic conditions with average annual rainfall of 600mm per annum. The rainy season last for only four months (June- September). The rest of the year is hot and dry with temperature ranging from 27 -33^{0}C in cooler period and 36 - 42^{0}C during the hottest period. The region is mostly covered by grasses and short trees and is suitable for sorghum, millet, maize, cowpea, groundnut, cassava and cotton (10).

Field Sampling and Sample Collection.

The survey was conducted in 49 farmers' fields from the six (6) States in North-Eastern Nigeria. During the survey, Cassava fields between three and six months after planting were sampled along accessible roads including highways, secondary and feeder routes at roughly equal intervals of 10 kilometers between sites (7, 29). The routes were selected to target the major cassava growing regions so as to capture the distribution of cassava mosaic disease within the study area (14). At each site, 30 plants were assessed along two diagonals of the sampled field. In each field, the coordinates were recorded using the global positioning system (GPS) and leaf samples were collected based on the type of symptom expression (mild, severe, very severe and symptomless) for DNA extraction and virus diagnosis (26, 22). Leaf samples were stored in herbarium during the field survey (18, 26). A total of 135 CMD symptomatic and asymptomatic leaf samples were collected and analysed at molecular biology laboratory, Kebbi State University of Science and Technology, Aliero and Environmental Institute for Agricultural Research, Burkina Faso.

CMD Incidence

The percent disease incidence was calculated by expressing in percent, the total number of infected plants per total number of plants sampled using the formula of (15, 34)

$$\text{CMD incidence (\%)} = \frac{\text{Number of plants with symptoms}}{\text{Total number of plants sampled}} \times 100$$

Infection Type

Infection types were categorized as "C" (cutting-borne) and "W" (whitefly-borne) infections. Where the lower first-formed leaves show symptoms, infection is assumed to be cutting-borne, while where only upper leaves show symptoms, infection is whitefly-borne (34).

Laboratory Analysis of Samples

DNA Extraction

Total DNA was extracted from one hundred and thirty-five (135) cassava leaf samples according to the protocol of (19), which was modified by (1). Extracted DNA was resuspended in 100µl of molecular grade water and stored at -20^{0}C prior to PCR.

DNA Quality Test and Quantification

Seventy (70) out of 135 extracted DNA samples were randomly selected for DNA quality test and quantification using spectrophotometer (Nano drop machine) prior to PCR running.

Testing of DNA Samples by PCR

The leaf samples were tested by PCR using JSP001/F (5'-ATGTCGAAGCGACCAGGAGAT-3') and JSP002/R (5'-TGTTTATTAATTGCCAATACT-3' primers for ACMV and JSP001/F and JSP003/R (5'-CCTTTATTAATTTGTCACTGC-3' primers for EACMV, to determine presence or absence of the virus in the field collected samples. A total reaction volume of 25 µl containing 2.5 of 10x PCR buffer, 0.1 100 mM $MgCl_2$, 0.5 of 2.5 mM dNTPs, 0.5 each of 10µM forward and reverse primers, 0.1 of Taq polymerase, 18. 8 of sterile distilled water and 2 of template DNA was used for the amplification. The cycling conditions were initial denaturation at 94 ^{0}C for 4 min followed by 35 cycles of denaturation at 94 ^{0}C for 45 s, annealing at 52 ^{0}C for 45 s and extension at 72 ^{0}C for 55 s, with a final extension of 10 min at 72 ^{0}C. Amplification was done using the standard thermal cycler, Gene Amp PCR System. The amplified DNA fragments were electrophoresed in 1% agarose gel stained with ethidium bromide and run at 80 volts for 30 minutes in x 0.5 Tris-Acetate-EDTA (TAE) buffer at pH 8. The gel was then, visualized under UV light and photographed using an Olympus digital camera

RESULTS

CMD Incidence

CMD incidence varied among the States but it occurred in all the six States surveyed in the North-eastern Nigeria at an average of 44.45%. It was most prevalent in Gombe (73.90%) followed by Bauchi (62.70%) while Yobe had the lowest disease incidence (21.11%). Thirty three percent (33%) i.e (2/6) of the States surveyed had CMD incidence greater than 50%. (Table 1)

CMD Infection Type

CMD infection was categorized as Whitefly-borne and Cutting-borne

infections. The percent incidence due to whitefly infection was higher (41.71%) than cutting-borne infection (2.74%), giving an overall average CMD incidence of 44.45% in the study area (Table 1). Gombe had the highest whitefly-borne incidence (73.90%), followed by Bauchi (62.70%) with the lowest (21.11%) recorded in Yobe. Cutting-borne infection was highest in Taraba (11.11%) than in Borno (3.33%) and Adamawa (2.00%) and none was observed in Bauchi, Yobe and Gombe (Table 1).

Table 1: CMD Incidence across the States of North eastern Nigeria.

State	Whitefly infection (%)	Cutting infection (%)	Total incidence (%)
Bauchi	62.70	0.00	62.70
Yobe	21.11	0.00	21.11
Gombe	73.90	0.00	73.90
Borno	31.11	3.33	34.44
Adamawa	27.20	2.00	29.20
Taraba	33.22	11.11	44.33
Total	250.24	16.44	265.68
Mean±SE	41.71± 3.49	2.74 ± 2.13	44.45±5.62

Virus Diagnosis by PCR

From the PCR diagnosis of 135 DNA extracts analyzed, 104 (77.2 %) gave positive results out of which 71 (52.6 %) had only ACMV, 19 (14.1 %) had only EACMV and 14 (10.4 %) had both EACMV and ACMV viruses in dual infection. ACMV was more pronounced in Bauchi 25 (35.2%) followed by Taraba (28.2%) and less in Borno (5.6%). EACMV was also more prevalent in Bauchi (47.4%) followed by Taraba (36.8%), less in Adamawa (5.3%), none in Gombe and Borno states 0(0.0%) each. Bauchi and Taraba had the highest dual infections (64.3%) and (28.6%) respectively while Yobe, Gombe and Borno had none (Table 2).

Table 2: Occurrence of Cassava Mosaic Geminiviruses in the North eastern States, Nigeria.

State	Number of samples (%)	Cassava Mosaic Geminiviruses		
		ACMV (%)	EACMV (%)	ACMV+EACMV (%)
Bauchi	39 (28.9)	25 (35.2)	9 (47.4)	9 (64.3)
Yobe	17 (12.6)	6 (8.5)	2 (10.5)	0 (0.0)
Gombe	15 (11.1)	8 (11.3)	0 (0.0)	0 (0.0)
Borno	14 (10.4)	4 (5.6)	0 (0.0)	0 (0.0)
Adamawa	12 (8.9)	8 (11.3)	1 (5.3)	1 (7.1)
Taraba	38 (28.1)	20 (28.2)	7 (36.8)	4 (28.6)
Total (%)	135 (100)	71 (52.6)	19 (14.1)	14 (10.4)

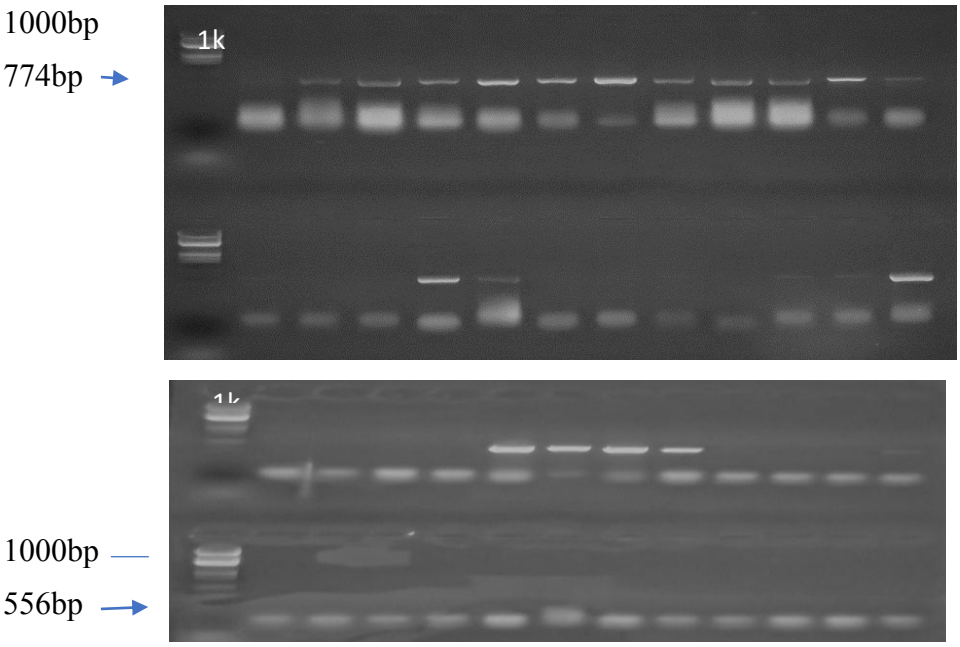

Figure 1. PCR amplification of coat protein of ACMV (774 bp) and EACMV (556 bp) from cassava samples using specific primers JSP001/2 and JSP001/3, respectively and a DNA 1Kb Ladder

DISCUSSION

This study constitutes the most current survey of Cassava mosaic disease in the North East, Nigeria. It was carried out between January and September, 2017, to determine the current status of Cassava mosaic disease in the region. The disease occurred in all the States surveyed, giving an overall incidence of 44.5%. This (44.5%) is lower than was reported from Zambia (8) and Cote d'Ivoire (37) with 57.4% and 100% disease incidences respectively but was high than that of Democratic Republic of Congo (29) with (38.4%) incidence. Gombe and Bauchi have the higher CMD incidences (73.9% and 62.7%) respectively. The higher incidence may be due to climatic conditions such as higher rainfall and air humidity which may lead to the increase in number and activity of the whiteflies, the vector for CMD dissemination. Similarly, the continuous use of the local cultivars could pose a threat to the crop should a more virulent virus strains or species emerged due to recombination or introduced into the area unless interventions in the form of introduction of resistant varieties and phytosanitation are practiced (9, 17).

Whitefly-borne infections were more frequent in all the States (41.71%) than cutting-borne infections (2.74%). This is in contrast to what was reported in Democratic Republic of Congo where cutting borne infections (82%) was higher than whitefly-borne infections with 18% incidence (28). Gombe had the highest whitefly incidence (73.9%) while Yobe had the lowest (21.11 %). Taraba had the highest cutting borne incidence (11.11%) followed by Borno (3.33%). These results show that Cassava mosaic disease (CMD) in North eastern Nigeria is mostly being spread by whitefly vectors rather than the use of infected cassava planting materials.

It is apparent from the PCR results that infections from African Cassava mosaic virus (ACMV) (52.6%) were more common compared to the infections caused by East African Cassava mosaic virus (EACMV) (14.1%). Previous studies (6) have also reported high frequency of single infections of ACMV compared to EACMV. Similarly, dual infections (ACMV+EACMV) is lower than the single infection of either ACMV or EACMV with percent incidence of 10.4%. This could be due to farmers leaving out severely infected plants when selecting cuttings for planting and various campaigns and training organized for farmers and extension workers in northern Nigeria (4, 5). The occurrence of dual infections in

some of the States surveyed might be fueled by movement of infected cassava planting materials which were not tested for virus presence. Bauchi and Taraba had highest percentage of occurrence of both single infections and dual infections which could be due to the high number of Cassava fields which may result in the accumulation of high number of whitefly vector in the States. The occurrence of ACMV and EACMV in dual infected plants and single infection (EACMV) in most of the States surveyed indicates that the prevalence of the two viruses (ACMV and EACMV) is wide spread. These findings agree with the previous studies (30) which reported the presence of the two viruses with ACMV being more prevalent than EACMV and dual infections of the two virus species.

CONCLUSION

In conclusion, our study has revealed that Cassava mosaic disease is widely spread in most of the Cassava growing areas of north eastern states of Nigeria, with the presence of ACMV in single infections in all the States and EACMV single infections in most of the States. Similarly, dual infections of the two viruses was established in Bauchi, Adamawa and Taraba. The study demonstrates that ACMV is widely distributed and it also highlights the urgent need for more information. The results also show that CMD infection is predominantly caused by whiteflies with relatively lower cutting borne infections.

The high CMD incidence recorded during the survey is of great concern which indicates the needs for more information as well as provision of management measures with regard to the disease in the study area. There is therefore the need to sensitize the farmers regarding the effects of CMD and means of obtaining the planting materials. Similarly, more working institutions (tissue culture laboratories unit and Cassava seed multiplication centers) should be established to test the Cassava cuttings before giving them out to farmers. Continuous monitoring of Cassava Mosaic Viruses and whiteflies population is also required to provide appropriate management strategies of the disease. Further studies should focus at characterizing the Cassava virus diversity by sequencing for thorough understanding of the existing types of virus strains in study area. Pre-emptive laboratory investigations are also needed to make sure Cassava brown streak viruses (CBSVs), which are the biggest Cassava threats in East and Central Africa are not present in West Africa.

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