DISTRIBUTION OF *CASSAVA MOSAIC GEMINI VIRUSES* IN TARABA STATE, NORTH EASTERN NIGERIA.

[a]Abubakar, [b]M. Mohammed I.U&[c]Mohammed, M.T.
[a]Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero.
[b]Department of Crop Science, Faculty of Agric,Kebbi State University of Science and Technology, Aliero.
[b]Department of Crop Protection, Faculty of Agriculture, Bayero University, Kano (BUK), P.M.B. 3011, Kano, Kano State, Nigeria
E-mails: abubakarm431@gmail.com, magajimohammed2015@gmail.com

Abstract
*Cassava mosaic begomoviruses*are among the most important biotic agents affecting cassava limiting the production potential of the crop in Northern Nigeria. A survey was conducted in five Districts of Taraba State, Nigeria to determine the cassava begomovirus strains that caused cassava mosaic disease in the study area. At each survey site, up to thirty plants were assessed for CMD symptom severity, incidence and whitefly population and in each field, leaf samples were collected for virus testing. Deoxyribonucleic acid (DNA) extracts from these leaves were tested for ACMV, EACMC and EACMV-Ug in a uniplex polymerase chain reaction (PCR) assay. The results revealed that cassava mosaic disease incidence was highest in Zing (83.33%) and lowest in Ardokola (36.67%). The disease symptom severity was generally low. It was also highest in Zing (3.11) and lowest in Sunkani (1.42). Adult whitefly (*Bemisiatabaci*) population averaged 33.24. It was highest in Zing (53.45) and lowest in Ardokola (12.42). Out of the 66 samples tested, African cassava mosaic virus (ACMV) was present in 48.48%, East African cassava mosaic virus (EACMV) in 25.76%, and both ACMV and EACMV in 13.64%; 12.12% of the samples analysed were negative to all the viruses tested. None of the samples was tested positive to the East African cassava mosaic virus-Uganda (EACMV-Ug). The results highlight the need for the implementation of control measures including phytosanitary measures with utilization of CMD-free materials for planting and adoption of resistant varieties.
Keywords: Taraba, Incidence, Whitefly, Gemini viruses, Polymerase chain reaction.

INTRODUCTION

Cassava (*Manihotesculenta*Crantz, family *Euphorbiaceae*) is the third largest source of carbohydrates in the world and an important food staple crop in sub-Saharan Africa (Hillocks *et al*., 2015). The starchy tuberous roots are a source of food and income for more than 800 million people in Africa, Asia, and Latin America. Africa contributes more than 56% to the world's production (262.6 million tons) (FAO, 2014). Cassava is moving towards an industrialized system in which plant material is used for a variety of products including starch, flour, and animal feed ((Legg *et al*., 2015). The demand for cassava and cassava-based foods is increasing in the country. However, productivity at 6.7 t/ha is very low compared with the average yield of 9.8 t/ha in Africa. Pests and diseases, especially cassava mosaic disease (CMD) caused by whitefly transmitted *Begomoviruses*(family *Geminiviridae*), are among the major factors for low yields. CMD is known to seriously decrease yields and the effects are further exacerbated by the widespread cultivation of susceptible landraces (Alabi*et al*., 2011)

Nine different begomovirus species, commonly referred as *Cassava mosaic begomoviruses*(CMBVs), have been identified in the CMD aetiology in different regions of Africa (Alabi*et al*., 2011; Harimalala*et al*., 2012; Tiendrébéogo *et al*., 2012). Of the various CMBVs, *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), and *East africancassava mosaic Cameroon virus* (EACMCV) are known to be widely prevalent in sub-Saharan Africa (Patil and Fauquet, 2009). Several strains of CMBVs have also been identified; most notable of these is EACMV-Uganda (EACMV-Ug) which was responsible for the devastating pandemic in East Africa in the 1990s (Legg *et al*., 2006). All these viruses are vectored by whitefly,*Bemisiatabaci*Gennadius (Hemiptera: Aleyrodidae), and also spread through the cuttings used routinely for vegetative propagation (Legg *et al*., 2011).

The viruses exhibit diverse infection dynamics in terms of symptom expression, progression, recovery, severity, and as well as host range (Bull *et al*., 2007; Patil and Fauquet, 2009). 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 (Stanley *et al*., 2004), 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 (Stanley *et al*., 2004). Co-infections of EACMV and ACMV in cassava have been reported in Zambia, Nigeria and Tanzania (Chikoti*et al*., 2013). This study was conducted to provide information on the incidence of Cassava mosaic begomoviruses and the severity of cassava mosaic disease in the study area so as to contribute to the development of appropriate control measures.

MATERIALS AND METHODS

Study Areas

Nigeria's land stretches from latitude 4°N to 14°N and from longitude 3°E to 14°E. Of this area, 71 million ha (77%) are considered cultivable; about 32 million ha (45% of the total cultivable land areas) are cultivated. Annual rainfall ranges from 4000mm in the coastal areas to about **500mm** in the far north (Adenijiet *al*., 2009). The temperature ranges from 26^{o}C coastal regions to 37^{o}C (Douglas, 2004).Taraba state, Nigeria stretches across Northern Guinea - climatic zones of the country on latitude 8° 00' 00'' N and longitude 10° 30'0. 00'' E with average annual rainfall of 600mm per annum. The rainy season last for only five months (May-September) in most of the regions while the rest of the year is hot and dry with temperatures 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, rice millet, maize, cowpea, groundnut, cassava and cotton (Sowunmi and Akintola, 2010).

Field Survey and Sample Collection

The survey was conducted in the five districts of Taraba State, Nigeria. A total of 33 cassava fields (farms) aged 3 – 6 months were sampled in the study area. The sampling was in farms along the major roads, secondary and feeder routes of the farmers' fields (Omongo*et al.*, 2007; Marcel *et al*., 2012). In each field, 30 plants were assessed along two diagonals of the sampled field. Information about the field such as regional/location names, crop age and cultivar name were all sought from the farmer. Field coordinates were recorded using the global positioning system (GPS). Plants were assessed for CMD incidence and severity based on the type of symptoms (incidence) and degree of symptom expression (severity) as mild, severe, very severe and symptomless. Leaf samples from each severity class were collected per field and preserved in herbarium specimen (Chickoti*et al*., 2015) for DNA extraction and virus diagnosis. A total of 66 CMD symptomatic and asymptomatic leaf samples were collected and analyzed at molecular biology laboratory, Kebbi State University of Science and Technology, Aliero.

Cassava Mosaic Disease (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 Sseruwagi*et al*. (2009) and Mohammed *et al*. (2012):

$$\text{Disease incidence (\%)} = \frac{\text{No.of plants with symptoms}}{\text{Total no. of plant sampled}} \times 100$$

Cassava Mosaic Disease (CMD) Severity

CMD severity was expressed using an arbitrary scale of 1 – 5, indicating the extent of symptom development (IITA, 1990; Hillocks *et al*., 2015; Maruthi*et al*., 2014; Mohammed *et al*., 2016) as follows: 1 = Symptomless plants, 2= Mild chlorotic patterns affecting most of the leaves, 3 = Pronounced chlorosis on most leaves with narrowing and distortion of lower one-third of the leaflets, 4 = Severe Chlorosis and distortion of two-third of most of leaves and general reduction of leaf size and some stunting and 5 = very severe (severe chlorosis, reduction of leaves, plant stunting, leaf distortion and dieback).

Whitefly Population

Whitefly (*Bemisiatabaci*) numbers was counted on the top five fully-expanded apical leaves and the 14th leaf of the tallest shoot respectively for 5 of the 10 plants sampled per field and the totals is recorded separately (Sseruwagi*et al*., 2009).

Deoxyribonucleic Acid (DNA) Extraction from Cassava Leaf Samples

Total DNA was extracted from one hundred and thirty five (94) cassava leaf samples according to the protocol of Lohdi*et al*. (1994), which was modified by Abarshi*et al*. (2012). Extracted DNA was resuspended in 100µl of molecular grade water and stored at -20^{0}C prior to PCR.

Deoxyribonucleic Acid (DNA) Quality Test and Quantification

Seventy (35) out of 66 extracted DNA samples were randomly selected for DNA quality test and quantification using spectrophotometer (NanoDrop 2000C) prior to PCR running.

Analysis of Cassava Samples by PCR

Cassava leaf samples were analysed by PCR using JSP001/F (5'-ATGTCGAAGCGACCAGGAGAT-3'), JSP002/R (5'-TGTTTATTAATTGCCAATACT-3' primers for ACMV and JSP001/F and JSP003/R (5'-CCTTTATTAATTTGTCACTGC-3' primers for EACMV and Uv-AL1/F1 (5'-TGTCTTCTGGGACTTGTGTG-3'), ACMV-CP/R3 (5'-TGCCTCCTGATGATTATATGTC-3') for EACMV- Ug to determine presence or absence of the virus in the field collected samples as shown in the reaction mixture below: 0.1 µl $Mgcl_2$ (100 Mm), 2.5 µl PCR buffer (10x), 18.8 µl SDW, 0.5 µl dNTPs (2.5 Mm), 0.5 µl JSP001/F (10 µM), 0.5 µl JSP002/R (10 µM ACMV), 0.5 µl JSP003/R (10 µM EACMV), (10 µM EACMV-Ug), 0.1 µl of 5 U/ µl Taq polymerase and 2.0 µl of the DNA template.The viral DNA was amplified using the standard thermal cycler, Gene Amp PCR System with the conditions below: initial denaturation at 94^{0}C, 1 cycle for 4 minutes, final denaturation at 94^{0}C, 1 cycle for 45seconds, annealing at 52^{0}C, 35 cycles, 45 seconds, initial extension at 72^{0}C, 1 cycle 55 seconds and final extension at 72^{0}C, for 10 minutes. The amplified DNA fragments were electrophoresed in 2% agarose gel stained with ethidium bromide and run at 10 volts for 30 minutes in x 0.5 Tris-Acetate-EDTA (TAE) buffer at pH 8. The gel was then visualized under UV light (transmillinator) and photographed using an Olympus digital camera with Digi Doc-gel imaging system.

Data Analysis

The data generated during the survey were processed and subjected to descriptive statistics using means, percentages, and standard error so as to provide summary description of the subject using descriptive statistical tools such as tables.

RESULTS

Cassava Mosaic Disease Incidence

Cassava Mosaic Disease is present in all the five (5) Districts surveyed in Tarab State, Nigeria with an average of 58.67%. It was most prevalent in Zing (83.33%) followed by Sunkani (73.33%) while Ardokola had the lowest disease incidence (36.67%). Sixty percent (60%) i.e. (3/5%) of the Districts surveyed had CMD incidence greater than 50% (Table 1).

Cassava Mosaic Disease Severity

CMD severity ranged from healthy (1.42) to very severe (3.11) with an overall mean score of 2.20. It was highest in Zing (3.11) and lowest in Sunkani district (1.42) (Table 1)

Whitefly Population

The mean number of adult whitefly was generally high, with an average of 33.24 adult whiteflies per plant. Zing had the highest number of adult whiteflies (53.45), followed by Balli (42.73 while Ardokola had (12.42), being the lowest (Table 1).

Polymerase Chain Reaction (PCR) -Based Detection of Viruses

PCR assays with oligonucleotide primers specific to ACMV, EACMV and EACMV-Ug were used to detect *Cassava mosaic begomoviruses* in the leaf samples collected from the field. From the diagnosis of 66 DNA extracts analysed, 58 (87.88 %) gave positive results out of which 32 (48.48 %) had only ACMV, 17 (25.76 %) had only EACMV and 9 (13.64 %) had both EACMV and ACMV viruses in dual infection. ACMV was more pronounced in Ardokola (28.13%) and least in Balli (12.5%). EACMV was more prevalent in Sunkani (29.41%) followed by Ardokola and Lau (23. 53%) each, while Zing had the least (5.66%). Zing had the highest dual infections (44.44%), less in Lau (2.22%) while Balli and Sunkani had none (0.0%) (Table 2)

Table 1: Parameters for CMD across Taraba State, North eastern Nigeria

Districts	Whitefly number	Severity (1-5)	Incidence (%)
Ardokola	12.42	2.15	36.67
Balli	42.73	1.75	43.33
Lau	38.6	2.58	56.67
Sunkani	18.9	1.42	73.33
Zing	53.45	1.11	83.33
Total	166.20	11.01	293.33
Means ± SE	33.24 ± 6.81	2.20 ± 0.27	58.67 ± 7.85

Table 2: Incidence of Cassava Mosaic Gemini-Viruses in Taraba State, Nigeria

		Gemini Viruses of Cassava			
Districts	Number of samples (%)	ACMV (%)	EACMV (%)	ACMV+EACMV (%)	EACMV-Ug (%)
Ardokola	15 (22.73)	9(28.13)	4(23.53)	3 (33.33)	0 (0.0)
Bali	9 (13.64)	4 (12.50)	3(17.65)	0 (0.0)	0 (0.0)
Lau	12 (18.18)	5(15.63)	4 (23.53)	2 (22.22)	0 (0.0)
Sunkani	14 (21.21)	7(21.88)	5 (29.41)	0 (0.0)	0 (0.0)
Zing	16 (24.24)	8(25.00)	1(5.88)	4 (44.44)	0 (0.0)
Total (%)	66 (100)	32 (48.48)	17(25.76)	9 (13.64)	0(0.00)

Keys: ACMV= African Cassava Mosaic Virus, EACMV= East African Cassava Mosaic Virus and EACMV-Ug = East African Cassava Mosaic Uganda Virus

DISCUSSION

The results of this study revealed that CMD is an endemic problem in all Districts of Taraba state, Nigeria. The incidence was higher in most of the areas visited during the survey. The high incidence rates observed in various fields suggests that stem cuttings are the likely origin of the virus. Traditionally, farmers reuse as planting materials stems from their own farms which are often infected by viruses. This explains why CMD is widely disseminated and may be prevalent in areas where disease spread by vectors is limited. In addition, the two widely predominant cultivars (white and red Cassava varieties), were found to be highly susceptible to the viruses that cause CMD. the continuous use of these 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 (Kenneth, 2007). Most of the planting materials in the fields are already infected thus

creating a dearth of CMD-free material. This leads to the perpetuation of viruses through infected stems (N'zué*et al*., 2005). Previous studies have reported a similar situation with regard to CMD in several countries in sub Saharan Africa and suggest that symptoms depend on the virus species, strains, and mixed infections (Fauquet and Fargette, 1990; Harrison *et al*., 1997; Fondong *et al*., 2000; Pita *et al*., 2001a, b; Ogbe*et al*., 2003; Alabi*et al*., 2008b).

Analysis of infected cassava leaf samples confirmed the presence of ACMV and EACMV but not EACMV-Ug. ACMV was the most prevalent begomovirus infecting cassava in all the districts of the State. Similar observations were reported by Harrison *et al*. (1997) in Uganda and Karakacha (2000) in Kenya. ACMV and EACMV occur in infected plants in Africa either alone or as mixed infections of different combinations (Fondong*et al*., 2000; Berry and Rey, 2001; Ogbe*et al*., 2003; Were *et al*., 2004; *Bull et al.*, 2006). The proportion of single infections by EACMV was higher (25.76%) than co-infections with ACMV (13.64%). Sources of inoculum are naturally infected plants when used as planting materials in successive years and also other herbaceous hosts of begomoviruses (Alabi*et al*., 2008b). The majority of the new fields were planted with cuttings of plants harvested in previous fields, and probably infected. Also, the activities of insect vectors have an effect on CMD incidence and the transmission of begomoviruses (Patil and Fauquet, 2009).

Ogbe in his studies on begomoviruses on cassava in Nigeria (Ogbe*et al*., 2001) observed that EACMCV was found in the humid forest, derived/coastal and southern Guinea savannas. EACMV-Ug was not detected in this study. However, occurrence of this strain in Burkina Faso underscores the need for vigilance against its spread in the country (Tiendrébéogo*et al.*, 2009).Some samples from asymptomatic leaves were tested positive for viruses. This indicates that the absence of virus infection cannot be assumed from the absence of visual symptoms on leaves.

CONCLUSION

The study confirms the occurrence of two cassava mosaic begomoviruses, ACMV and EACMV, causing CMD infection in Taraba. ACMV and EACMV single infections were the most frequently occurring viruses in the plants infected by CMD than the dual infections of the two viruses. The high levels of disease incidence and severity found in the surveyed fields highlights the urgent need for more information. A higher whiteflies number were found in some of the fields visited but with less CMD incidence which confirms that the presence of whiteflies per field may not be an indication of possible infection with the virus. However, most of the states that have higher whiteflies number also have the highest mean severity and percent incidence of the disease.

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