

INCIDENCE OF CASSAVA MOSAIC DISEASE IN KEBBI STATE, NIGERIA

Mohammed, I. U. and Muhammad, A.

Kebbi State University of Science and Technology, Aliero

Corresponding Author: iumohammed74@gmail.com

SUMMARY

Cassava mosaic disease (CMD) is one of the most important and biotic constraints affecting cassava limiting the production potential of the crop in Northern Nigeria. This study was carried out to evaluate the incidence, severity and infection type of the disease in Kebbi State, Nigeria. A total of thirty-three (33) fields were surveyed and, in each field, thirty plants were assessed along two diagonals and symptomatic and non-symptomatic leaf samples were collected from the fields surveyed for laboratory analysis. The results of the study revealed that CMD incidence was highest in Gwandu (89%) and lowest in Bunza (43%). The disease symptom severity was generally mild. It was more severe in Gwandu and Jega (3) and lowest in Bunza (1). Cutting-born infection is most prevalent (60%) than whitefly-born infection (3%) in the Local Government Areas surveyed. Polymerase chain reaction (PCR) results using specific primers for *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV) detected single infections of ACMV and EACMV in 62% and 13% of the positive samples respectively. Co-infections of ACMV and EACMV were detected in 11% of the tested samples. Continuous monitoring of cassava mosaic viruses and whiteflies population is required to provide appropriate management strategies of the disease in the State.

Keywords: Kebbi, Severity, ACMV, EACMV, Polymerase chain reaction.

CASSAVA (*Manihot esculentus*, Crantz), belonging to Euphorbiaceae family, is a perennial which grows in tropical and sub-tropical areas of the world (23). It originated from tropical

America (Brazil) and was first introduced into Africa in the Congo basin by Portuguese traders around 16th Century (13). Cassava can grow to a height of 3 m. The stem has nodes

at intervals from which the leaves are produced. The leaves are large lobed, borne on a long, slender stalk joining a leaf. The color of leaves is dark green but, in some varieties, yellow or purple pigmentation may occur. Male and female flowers are found on the same plant (4). Cassava is known to grow in different environmental conditions. It is suited to warm humid lowland tropics and can be cultivated in most areas where the mean annual temperature exceeds 20°C with annual rainfall that varies between 500 mm and 8000 mm (9). Continuous light delays storage root formation, lowering yields and it is most productive when day length is up to 12 hours which falls between latitudes of 30° S and 30° N. Cassava tolerates a soil pH range from 4 to 9 (11).

The bulk of cassava grown in Africa is utilized as food in the form of fresh roots and processed products such as flour and fermented meal preparations (12). Furthermore, Cassava leaves are consumed as vegetables and are the source of proteins, vitamin A, B and C. They contain an average of 21% protein, which is considered high among non-leguminous plants (4). Cassava is a staple crop in many African countries including Zambia, Mozambique, Tanzania, Malawi, Democratic Republic of Congo (DRC) and Nigeria. In Nigeria, the average yield

is around 8.9 t ha⁻¹, which is below that of Africa at 11.1 t ha⁻¹ (10).

Cultivation of cassava in Africa and Nigeria in particular, is constrained by several biotic factors of which diseases are the most important. The diseases include cassava mosaic disease (CMD), cassava anthracnose disease (CAD), cassava bacterial blight (CBB) and cassava brown streak disease (CBSD). Cassava mosaic disease is caused by several distinct whitefly transmitted begomoviruses [African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV), East African cassava mosaic Cameroon virus (EACMCV), East African cassava mosaic Uganda virus (EACMV-Ug), 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)] (14). Among these viruses, cassava mosaic geminiviruses are the most economically important viruses of Cassava in Africa (14). In Cassava, losses in tuber yield due to diseases can be as high as 90% (17). Considering the crucial role of cassava to food security and hunger alleviation in Sub-Saharan Africa, there is a need to strategize and develop ways to mitigate cassava

yield losses caused by diseases, particularly viruses.

This study was carried out to assess the status of CMD in Kebbi State, North western Nigeria. This information would be of great benefit to Cassava growers, researchers especially breeders and virologists, as well as policy makers. It will help in identifying disease hot spot areas for deployment of clean planting materials to farmers in the State. The information will also help in guiding decisions in choosing areas suitable for Cassava seed multiplication.

MATERIALS AND METHODS

Study area

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 area) are cultivated. Annual rainfall ranges from 4000 mm in the coastal areas to about 500 mm in the far north. The temperature ranges from 26°C coastal regions to 37°C (8). Kebbi state, Nigeria stretches across Sudan agro-climatic zones of the country on latitude 12⁰.31°N and longitude 4⁰.50°E with average annual rainfall of 600 mm per annum. The rainy season lasts for only four months (June-September) while the rest of the year is hot and

dry with temperatures ranging from 27-33⁰C in cooler period and 36 - 42⁰C 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 (24).

Field sampling and sample collection

The survey was conducted in the five Local Government Areas (LGAs) of Kebbi State, Nigeria namely Argungu, Bunza, Gwandu, Jega and Zuru. A total of 33 cassava fields aged 3 – 6 months were sampled. The sampling was in cassava farms along the major roads, secondary and feeder routes of the farmers' fields (22). 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 local name of cultivar were sought from the farmer. Field coordinates were recorded using the global positioning system (GPS). Plants were assessed for CMD incidence based on the number of plants showing symptoms and severity based on the degree of symptom expression as mild, severe, very severe and symptomless. Leaf samples from each severity class were collected per field and preserved in form of herbarium specimen (7) for DNA extraction and virus diagnosis. A total of 165 CMD symptomatic and

asymptomatic leaf samples were collected and analyzed at Molecular Biology Laboratory, Kebbi State University of Science and Technology, Aliero, Nigeria.

Cassava mosaic disease 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 (25) and (18) as stated below.

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

plants sampled

Cassava mosaic disease severity.

Cassava mosaic disease severity was expressed using an arbitrary scale of 1 – 5, indicating the extent of symptom development (10; 9; 16; 17) 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).

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 (25).

DNA extraction from cassava leaf sample

Total DNA was extracted from 165 cassava leaf samples according to the protocol of (3), which was modified by (1). Extracted DNA was resuspended in 100µl of molecular grade water and stored at -20°C prior to PCR.

DNA quality test and quantification

Seventy (70) out of 165 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 Polymerase chain reaction (PCR)

Cassava leaf samples were analyzed 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 to determine presence or absence of the virus in the field collected samples in the reaction mixture below: 0.1 µl MgCl₂ (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), 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; initial denaturation at 94°C, 1 cycle for 4 minutes, final denaturation at 94°C, 1 cycle for 45seconds, annealing at 52°C, 35 cycles, 45 seconds, initial extension at 72°C, 1 cycle 55 seconds and final extension at 72°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 (transilluminator) and photographed using an Olympus digital camera with Digi Doc-gel imaging system.

Data analysis

The data generated during survey were processed and subjected to descriptive statistics using means, percentages, and standard error to provide summary description of the result.

RESULTS

CMD Incidence and Severity

Cassava mosaic disease occurred in all the five LGAs surveyed in Kebbi State of Nigeria with an average of 62.4 %. It was most prevalent in Gwandu (89 %) followed by Jega (70 %) while Bunza had the lowest disease incidence of 43 %. Eighty percent (80 %) of the surveyed LGAs had CMD incidence greater than 50 %. CMD severity ranged from healthy (1) to very severe (3) with an overall mean score of 2. It was highest in Gwandu and Jega (3) and lowest in Bunza (1) (Table 1).

CMD Infection Type.

CMD infection was categorized as whitefly - mediated and cutting - mediated infections. The percent incidence from cassava cuttings was higher (60%) than by whiteflies (3 %), giving an overall average CMD incidence of 62 % in the study area (Table 2). Gwandu had the highest cutting-borne incidence (85 %), followed by Jega (60 %) with the lowest (43 %) recorded in Bunza. Whitefly-borne infection was highest in Jega (10 %) than in Gwandu (3 %) and none was observed in Argungu, Bunza and Zuru (0 %) (Table 2).

Table 1: Parameters for cassava mosaic disease across Kebbi State, Nigeria

LGA	Mean number of whiteflies	Mean of symptom severity score	Infection (%) via whiteflies	Infection (%) via cuttings	Total incidence (%)
Argungu	27.63	2.00	0.00	43.33	43.33
Bunza	28.54	1.00	0.00	53.33	53.33
Gwandu	56.33	3.00	3.33	85.33	88.66
Jega	31.14	3.00	10.00	60.00	70.00
Zuru	40.93	2.00	0.00	56.67	56.67
Total	194.57	11.00	13.33	298.66	311.99
Means±SE	38.91±4.91	2.00±0.26	2.67 ± 1.74	59.73± 6.23	62.40±6.99

LGA = Local Government Area

Table 2: Distribution of cassava mosaic geminiviruses in Kebbi State, Nigeria.

LGA	Number of samples tested	Geminiviruses detected*		
		ACMV	EACMV	ACMV+EACMV
Argungu	16	10 (19)	2 (18)	3 (33)
Bunza	28	19 (36)	4 (36)	5 (56)
Gwandu	19	11 (21)	0 (0)	0 (0)
Jega	15	8 (15)	3 (27)	0 (0)
Zuru	8	5 (9)	2 (18)	1 (11)
Total (%)	86	53 (62)	11 (13)	9 (11)

LGA = Local Government Area; * = Percentage incidence is indicated in parenthesis

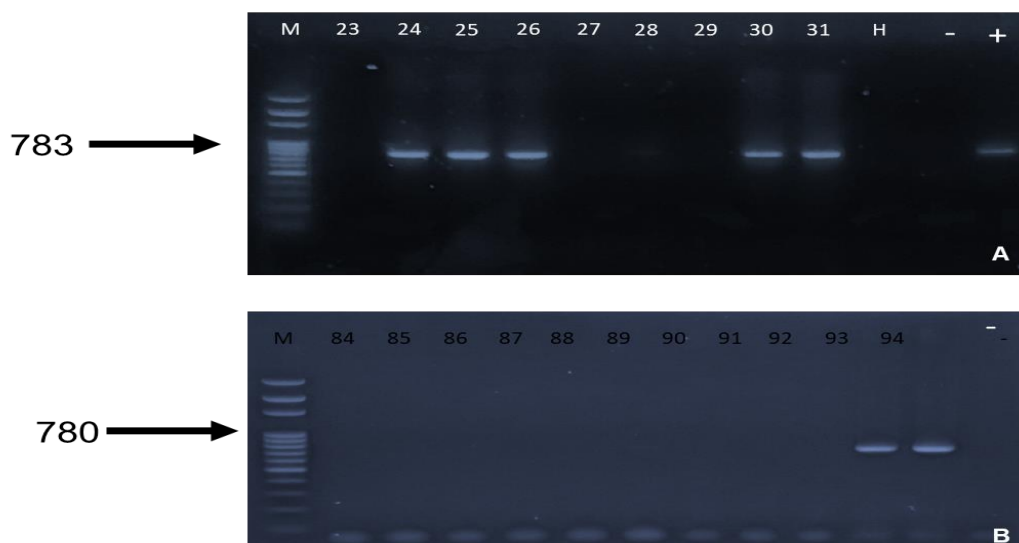


Figure 1: Amplification of ACMV (A) and EACMV (B) genomes using JSP001, 002 and 003 primers; H = DNA from a CMD-free plant, – = negative water control, and + = a known ACMV DNA; M = 1000 bp molecular size ladder (New England Biolabs, UK).

African cassava mosaic virus (EACMV) by PCR

Two viruses were detected using virus specific primers: JSP001F and JSP002R, JSP001F and JSP003R, which specifically amplified ACMV (783bp) and EACMV (780bp) respectively (Fig. 1a and b). No amplifications were obtained from DNA extracted from healthy plants.

DISCUSSION

The results of this study have shown that cassava mosaic disease (CMD) is widely distributed in all the LGAs surveyed in Kebbi State giving an overall incidence of 62%. The higher CMD incidences in Gwandu (89%)

and Jega (70%) may be due to climatic conditions such as higher rainfall and humidity which lead to the increase the CMD incidence. Similarly, susceptibility of the crop used in the study area may lead to higher CMD incidences. It was observed that only the white and red varieties, which are susceptible to the viruses, are predominantly cultivated in these areas. The fact that in these areas there are only few predominant cassava varieties (white and red). The continuous use of these varieties 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 (13).

Cutting-borne infections were found to be more prevalent in all the LGA than whitefly-borne infections. Gwandu had the highest cutting incidence (85%) while Bunza had the lowest (43%). These results showed that CMD in Kebbi State is mostly being spread by farmers rather than the whitefly. It was observed from this study that disease incidence of 62% was higher than was reported by (7) and (22) which reported 57% and 38% disease incidences in Zambia in the Democratic Republic of Congo respectively but was lower than was reported by (26) which reported 100% incidences in Côte d'Ivoire. PCR diagnostic results revealed that infections of African cassava mosaic virus (ACMV) (62%) were more common compared to the infections caused by East African Cassava mosaic virus (EACMV) (11%). Previous studies (3) have also reported high frequency of single infection of ACMV compared to EACMV. Similarly, frequency of mixed infection by ACMV+EACMV was lower than the single infection of either ACMV or EACMV with percent incidence of 11%. This could be due to farmers leaving out severely infected plants when selecting cuttings for planting and the various

campaigns and training organized for farmers and extension workers in northern Nigeria by both Program for Emerging Agricultural Researchers (PEARLs) and West African Virus Epidemiology (WAVE) funded by both Bill and Melinda Gates Foundation (BMGF).

The occurrence of dual infections in some of the LGAs surveyed except Bunza and Gwandu might be fueled by movement of infected cassava planting materials which probably were not tested for virus presence in the country. In this study, Argungu had the highest percentage occurrence of both single infections and dual infections of CMD viruses 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 state. The occurrence of ACMV and EACMV singly and in mixed infection dual infected plants and single infection (EACMV) in most of the LGAs surveyed indicates that the prevalence of the two viruses (ACMV and EACMV) is wide spread. These findings agree with the previous studies (22) which reported the presence of the two viruses.

Severe and mild CMD symptoms severity were observed in most of areas visited. CMD severity score was generally low with two (2) as the overall mean but was highest in

Gwandu (3) and lowest in Bunza (1). This is lower than the findings of (6) in Guinea where 3 was recorded as the mean severity score and what was reported by Tempo *et al.* (2017) with mean severity score of 4 on scale 1 to 5. It was observed from this study that the LGAs with the highest and lowest severity (Gwandu and Bunza) also had the highest and lowest percent incidences which indicates that there might be direct relationship between the disease severity and disease incidence. These results agreed with the previous studies (7; 22; 6) who reported the region with the highest severity to have the highest percent disease incidence. The high severity could be due to the dual infection of ACMV and EACMV strains which highlights the fact that the more the severity the more disease incidence and virus titer as was reported by (21).

CONCLUSION

In conclusion, this study has revealed that cassava mosaic disease is present in most of the cassava growing areas of Kebbi states, Nigeria. ACMV exists in single infection in all the LGAs and EACMV single infection in most of the LGAs except Gwandu.

REFERENCES

1. Abarshi, M.M., Mohammed, I.U., Jeremiah, S.C., Legg, J.P., Lava Kumar, P., Hillocks, R.J. and Maruthi, M.N. 2012. Multiplex RT-PCR assays for the simultaneous detection of both RNA and DNA viruses infecting cassava and the

Similarly, dual infections of the two virus strains (ACMV+EACMV) has been detected in Jega, Argungu and Zuru. The results also showed that CMD infection is predominantly caused by exchange of infected cuttings by farmers and relatively low whitefly-borne infections. Most of the states with higher whiteflies number also had the highest mean severity and percent incidence of the disease. There is need thus, there is need for more awareness creation on the effects of CMD and means of getting clean planting materials. Continuous monitoring of cassava mosaic begomoviruses (CMBs) and the other East African cassava virus disease CBSD are required to provide appropriate management strategies of cassava viruses in Nigeria.

ACKNOWLEDGEMENTS

Funding for this work was provided by the Bill and Melinda Gates Foundation and Department for International Development (DFID) Grant no. OPP1082413 “West African Virus Epidemiology (WAVE) for root and tuber crops” through a sub-grant from Université Félix Houphouët-Boigny (UFHB).

- common occurrence of mixed infections by two Cassava brown streak viruses in East Africa. *Journal of Virology Methods*. 179, 176-184.
2. **Adeniji, A.A., Ega, L.A., Akorodo, M.O., Ugwu, B.O. and Balugu, A.D. 2009.** Cassava development in Nigeria. A country case study towards a Global strategy for Cassava development Pp 5-6.
 3. **Alabi, O.J., Kumar, P.L. and Naidu, R.A. 2011.** Cassava mosaic disease: A curse to food security in Sub Saharan Africa. Online APS net features. Retrieved 12 January 2017 from <http://www.apsnet.org/publications/apsnetfeatures/Pages/cassava.aspx>.
 4. **Aloyce, C.R. 2013.** Development and evaluation of efficient diagnostic tools for Cassava mosaic and Cassava brown streak diseases, Pp 9-15 11.
 5. **Asare, P.A., Galyoun, I.K.A., Asare- Biediako, E., Sarfo, J.K. and Jetteh, J.P. 2014.** Phenotypic and molecular screening of Cassava Genotypes for resistance Cassava mosaic disease. *Journal of general and molecular virology*. 6(2): 6-18.
 6. **Bar, E.S., Bamkefa, B.A., Winter, S. and Dixon A.G.O. 2011.** Distribution and current status of Cassava mosaic disease and Begomoviruses in Guinea. *Article in AJRTC*. 9(1): 17-23.
 7. **Chikoti, P.C., Tembo. M., Chisola. M., Ntawarahunga. P. and Ndunguru. J. 2015.** Status of Cassava mosaic disease and whitefly population in Zambia. *African journal of Biotechnology*. 14: 2539-2546.
 8. **Douglas A, 2004.** Geographical, historical and political profiles of Nigeria. Pp 6-7.
 9. **Hillocks, R, J. Maruthi M., Kulembeka H., Jeremiah S., Alacho, F., Masinde, E., Ogendo, J., Arama, P., Mulwa, R., Mkamilom, G. and Kimata, B. 2015.** Disparity between leaf and root symptoms and crop losses associated with Cassava brown streak disease in four countries in eastern. *African Journal of phytopathology*. 64(2):86-93.
 10. **International Institute for Tropical Agriculture (IITA) 2016.** Laboratory manual for the Diagnosis of Cassava Mosaic Disease, Pp 25- 55.

11. **Irungu, J. 2011.** Prevalence and co-infections of Cassava with Cassava mosaic Geminiviruses and Cassava brown streak virus in popular cultivars in Western Kenya. M. Sc. dissertation. Pp 5-10.
12. **Kumar, P.L., Alabi, O.J., Akinbade, S.A., Maruthi, M.N., Naidu, R.A. and Legg, J.P. 2009.** Rapid single-step multiplex reverse transcription-PCR for the simultaneous detection of Cassava brown streak virus, African Cassava mosaic virus and East African Cassava mosaic virus in cassava. International Conference on Cassava Cultivation and Utilization in Central Africa Kisangani, RDC, 16 to 19th November 2009.
13. **Kenneth, G. M. 2007.** Epidemiology of Cassava mosaic disease and molecular characterization of Cassava mosaic viruses and their associated whitefly vector in South Africa, Pp 2-9.
14. **Lodhi, M. A., Ye, G. N., Weeden, N. F. and Reisch, B. 1994.** A simple and efficient method for DNA extraction from grapevine cultivars and Vitis species. *Plant Molecular Biology Reporter*.12:6-13.
15. **Legg, J.P., Jeremiah, S.C., Obiero, H.M., Maruthi, M.N. Ndyetabula, I., Okao-Okuja, G., Bouwmeester, H., Bigirimana, S., Tata-Hangy, W., Gashaka, G., Mkamilo, G., Alicai, T., and Lava Kumar, P. 2011.** Comparing the regional epidemiology of the Cassava mosaic and Cassava brown streak virus pandemics in Africa. *Virus Research* 159:161-170.
16. **Legg, J.P., Kumar, P.L., Makeshkumar, T., Tripathi, L., Ferguson, M., Kanju, E., Ntawuruhunga, P and Cuellar, W. 2015.** Cassava virus diseases: Biology, epidemiology and management. *Virus Research* 91:85-142.
17. **Maruthi, M.N., Bouvaine, S., Tufan., H. A., Mohammed, I. U. and Hillocks, R. J. 2014.** Transcriptional Response of virus infected Cassava and identification of putative sources of Resistance to Cassava Brown Streak Disease. *PLoS ONE*. 9(5): e96642.doi: 10.1371/journal.pone.0096642
18. **Mohammed, I.U., Ghosh S, and Maruthi, M.N. 2016.** Host

- and virus effects on reversion in Cassava affected by Cassava brown streak disease. *Plant pathology*. 65:593-600.
19. **Mohammed, I. U., Ghosh, S. and Maruthi, M.N. 2017.** Generating virus free Cassava by in vitro propagation with chemical and heat treatment. *African Journal of Biotechnology*. 16, 1551-1560.
 20. **Ndunguru, J., Fofana, B., Legg, J.P., Challappan, P., Taylor, N., Aveling, T., Thomson, G. and Fauquet, C. 2008.** Two novel satellite DNAs associated with bipartite cassava mosaic begomoviruses enhance symptoms and capable of breaking high virus resistance in Cassava landraces. In: Proceeding of the First Scientific Meeting of the Global Cassava Partnership (GCP-I) Ghent, Belgium, July 21 - 25, 2008.
 21. **Ntawurahanga, P., Okao-okuja, G. Bembe, A., Obambi, M. Armand, J. C. and Legg, J.P. 2007.** Incidence and diversity of Cassava mosaic disease in the Republic of Congo. *African crop science journal*. 15: 1-9.
 22. **Obiero, H., Akhwale, M., Okao-Okuja, G. and Asiimwe, P. 2007.** Monitoring and diagnostic survey of Cassava mosaic disease in Western Kenya. Pp 1-13.
 23. **Omongo, C.A., Kawuki, R., Bellotti, A.C., Alicai, T., Baguma, Y., Maruthi, M.N., Bua, A. and Colvin, J. 2012.** African Cassava Whitefly, *Bemisia tabaci*, resistance in African and South American cassava genotypes. *Journal of Integrated Agriculture*. 11:327-336.
 24. **Sangare, M., Atcham, T., Olivier, K., Bagui, I., Traore, A., Jeremie, B. and Thouakesseh, Z. 2015.** Classification of African mosaic virus infected Cassava leaves by the use of multi spectral imaging. *Optics and phonic journals*. 5: 261- 272.
 25. **Sowunmi, A. and Akintola, J.O. 2010.** Effect of climatic variability on Maize production in Nigeria. *Journal of Environmental and Earth sciences*. 2(1): 19-30.
 26. **Sseruwagi, P., Legg J.P., Maruthi M.N., Colvin J., Rey M.E.C. and Brown, J.K. 2009.** Genetic diversity of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) populations and presence of the B biotype and a non-B biotype that can induce silverleaf symptoms in

- squash in Uganda. *Annual Applied Biology*. 147: 253-265.
27. **Toualy, M.N.Y., Akinbade, S., Koutoua, S., Diallo, H. and Kumar, P.L. 2014.** Incidence and Distribution of Cassava mosaic begomoviruses in Côte d'Ivoire. *International Journal of Agronomy and Agricultural Research*, 4(6): 131-139.