academicJournals

Vol. 8(16), pp. 1350 -1353, 2 May, 2013
DOI: 10.5897/AJAR11.569
ISSN 1991-637X ©2013 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Molecular detection of two cassava *Begomoviruses* in some parts of Southern Nigeria

Eni A. O.* and Fasasi D. K.

Department of Biological Sciences, College of Science and Technology, Covenant University, KM 10 Idiroko Road, Canaanland, P. M. B. 1023 Ota, Ogun State, Nigeria.

Accepted 24 April, 2013

Cassava mosaic disease (CMD), caused by an array of is the most economically important viral disease of cassava in sub-Saharan Africa. The most frequently reported in West Africa are African cassava mosaic virus (ACMV) and East African cassava mosaic Cameroon virus (EACMCV). In this study, 42 cassava leaves and 30 symptomatic weeds belonging to the Asteraceae, Cucurbitaceae and Leguminosae families were collected from backyard gardens in Edo, Ondo, Anambra, and Delta States in 2009. Deoxyribonucleic acid (DNA) extracts from these leaves were tested for ACMV and EACMCV in a multiplex polymerase chain reaction (PCR) assay. The PCR primers used were designed to amplify the replicase regions of DNA-A components of both viruses. Most of the cassava plants within the survey area were either symptomless or showed mild symptoms. ACMV was detected in 16% of cassava leaves from Edo State but not in any of the cassava leaves from the other three states. One weed sample each from Edo State (5.56%) and Ondo State (10%) were also positive for ACMV. EACMCV was not detected in any of the samples tested. The low virus occurrence observed from PCR results and the observed low incidence of the CMD characteristic mosaic symptoms on cassava leaves in the states sampled may be attributed to the use of CMD resistant or tolerant cassava varieties, and may be a result of the massive distribution of virus resistant cassava cuttings to these States by the International Institute of Tropical Agriculture (IITA).

Key words: African cassava mosaic virus (ACMV), East African cassava mosaic Cameroon virus (EACMCV), multiplex polymerase chain reaction (PCR).

INTRODUCTION

Cassava (*Manihot esculenta*) is one of the leading food and feed plants of the world, particularly in Africa (FAO, 2008; Nassar and Ortiz, 2007). It is grown mainly for its enlarged starch-filled root, which is used for human consumption and, commercially, for the production of animal feed and starch-based products (O'Hair, 1990). Young tender cassava leaves are consumed as vegetables in many regions in Africa as a source of protein, vitamins and mineral salts (Legg and Fauquet, 2004).

Cassava mosaic disease (CMD) is the major constraint to cassava production in Africa and is caused by eight distinct of the family *Geminiviridae*, commonly referred to as Cassava mosaic (CMGs) (Fauquet and Stanley, 2003; Thresh and Cooter, 2005; Fauquet et al., 2008). CMD results in stunting and severe reduction in the yield of the desired tuberous cassava root and is thus a production threat to cassava which feeds over 200 million people in sub-Saharan Africa. CMD has been reported as occurring at varying levels of incidence throughout the cassava belt of Africa (Ogbe et al., 2003b; Thresh et al., 1997). For several years after African cassava mosaic virus (ACMV)

*Corresponding author. E-mail: angelaeni@gmail.com. Tel: +234 086 393 9991.

was confirmed as the causal agent of CMD, it remained the only known causal agent of CMD in Nigeria until the 1990s when East African cassava mosaic Cameroon virus (EACMCV) and several variants of the EACMV were diagnosed as additional causative agents (Ogbe et al., 2003a; 2003b; Ariyo et al., 2005; Ogbe et al., 2006; Alabi et al., 2008b).

Cassava plants infected with CMGs express a range of symptoms depending on factors like the virus strain or species, environmental conditions and the susceptibility of the cassava host (Legg and Thresh, 2003). CMD-affected plants are stunted with conspicuous foliar symptoms such as mosaic and chlorosis interfering with the photosynthetic ability of the plant to produce food for storage in the roots, thus, infected plants produces greatly diminished or no tuberous roots.

This study reports the testing of cassava and symptomatic weeds collected from backyard gardens in some states in Southern Nigeria, for ACMV and EACMCV.

MATERIALS AND METHODS

Sample leaves from cassava plants showing the CMD characteristic mosaic symptom were collected from farms in Edo, Delta, Ondo and Anambra States in July, 2009. A few non-symptomatic cassava leaves were also collected. Leave samples were also collected from symptomatic *Chromolaena odorata* (L.) King and Robinson, *Centrosema pubescens* Benth., *Senna alata* (Linn.) Roxb., and *Cucurbita* spp weeds within the vicinities of the sampled gardens, to ascertain their role as alternate/reservoir hosts for the viruses. Leaf samples were kept on ice while in the field and processed the same day by splicing and drying (rapidly) in anhydrous calcium chloride. Total deoxyribonucleic acid (DNA) was extracted from cassava and weed samples as previously described by Dellaporta et al. (1983).

The polymerase chain reaction (PCR) mixture consisted of 2.2 × PCR buffer, 0.25 mM of each dNTP, 0.533 µmol of each of the primers and 1 U of Taq DNA polymerase (Promega, Madison W1). The primers used for the multiplex PCR were CMBRep/F (5'-CRTCAATGACGTTGTACCA-3'), ACMVRep/R (5'-CAGCGGMAGTAAGTCMGA-3') and EACMVRep/R (5'-GGTTTGCAGAGAACTACATC-3') (Alabi et al., 2008b). Cycling conditions consisted of one cycle of denaturation at 94°C for 1 min, annealing at 48°C for 30 s and extension at 72°C for 1 min, followed by 36 cycles of denaturation at 94°C at 1 min, annealing at 48°C for 30 s and extension at 72°C for 1 min and a final extension at 72°C for 5 min.

The PCR amplified products were resolved by agarose gel electrophoresis and visualized under ultraviolet (UV) light using a gel documentation system. A 100 bp DNA molecular weight marker (Promega Corporation, Madison, W1) was run in each gel as a reference to estimate the size of the virus-specific DNA band in the PCR amplified products.

RESULTS

A total of 42 cassava leaves and 30 weeds were collected for virus testing. Seven (7) of the cassava leaves were non-symptomatic, while 35 were symptomatic. All the weeds tested showed leaf mosaic symptoms, and they were tested to determine their role as alternative host for the viruses. The distribution of leaf mosaic both on cassava and weeds across the states is shown in Figure 1.

The expected amplicon sizes for ACMV and EACMCV are 368 and 650 bp, respectively. Only 8.33% of the 72 samples produced the expected amplicon size of 368 bp for ACMV. Four of the positive samples were cassava leaves from Edo state; three of these were symptomatic, while one was non-symptomatic (Table 1). One *C. pubescens* leaf sample from Edo State and one *S. alata* sample from Ondo State tested positive for ACMV. None of the samples from Delta and Anambra States tested positive to ACMV, and EACMCV was not detected in any of the samples tested in all the States.

DISCUSSION

This study describes our quest to determine the occurrence of ACMV and EACMCV in cassava and associated weeds in backyard gardens in some parts of Southern Nigeria. Most cassava plants in the gardens surveyed were non-symptomatic. The high incidence of uninfected cassava plants observed in this study differs from reports from previous surveys in Nigeria where high incidence of CMD symptoms were reported in cassava fields (Ogbe et al., 2003a, 2003b, 2006). This difference may be due to the massive distribution and use of healthy, resistant, and/or tolerant cassava varieties by the farmers in the sampled areas (Manyong et al., 2000). This clearly indicates that increasing farmers' awareness on the cause and nature of a disease and possible control measures ensures their participation in the eradication of the diseases. The control of CMD is achieved mainly by the use of resistant/tolerant varieties and by employing adequate phytosanitry techniques of destroying diseased plants and by the use of healthy stems.

The positive detection of ACMV in some of the weeds tested confirms previous reports that weeds serve as alternate host for (Monde et al., 2010; Alabi et al., 2008a; Ogbe et al., 2006) and further buttresses the role of phytosanitation in the control of ACMV. This information is also of epidemiological importance because infected weeds may serve as virus reservoirs and inoculum foci.

Although, most positive samples were symptomatic, the detection of ACMV in one of the non-symptomatic cassava leaves indicates the possibility of latent infection. This phenomenon had been previously reported during field surveys in 2002 and 2003 (Ogbe et al., 2006), and further highlights the need for laboratory certification of cassava planting materials.

The negative EACMCV result obtained for all the leaves tested may indicate either the absence of the virus in the fields sampled, or an extremely low incidence. The high incidence of negative symptomatic cassava leaves

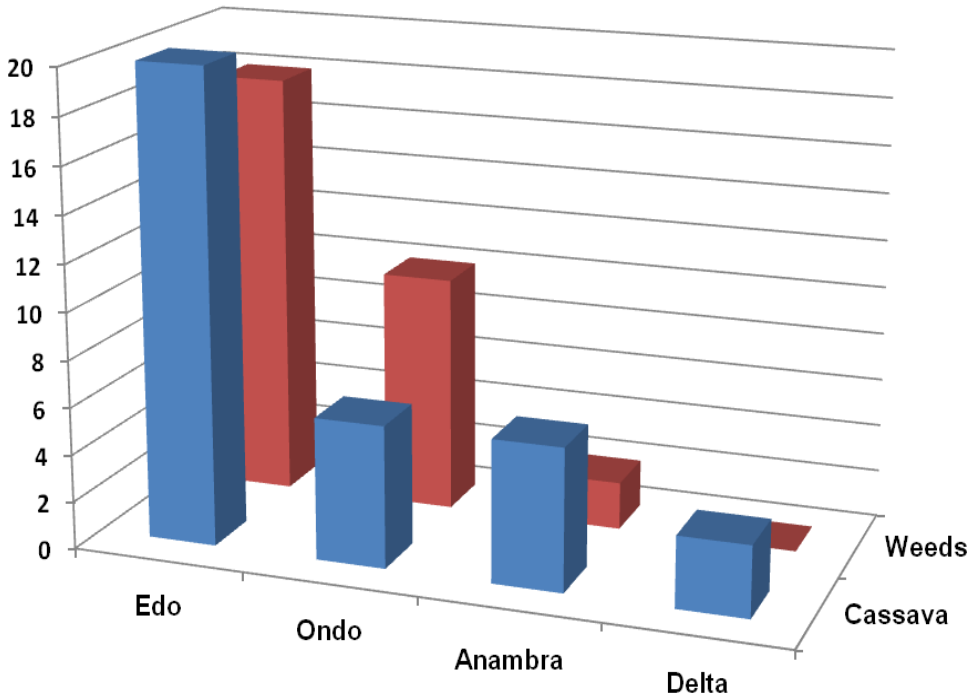

Figure 1. Distribution of leaf mosaic symptom on cassava leaves and weeds collected from Edo, Ondo, Anambra, and Delta States, Nigeria.

Table 1. Summary of cassava and weed samples collected and the result of multiplex PCR testing for ACMV and EACMCV in the surveyed samples from Edo, Delta, Ondo and Anambra states, Nigeria.

State	Plants	Number collected		ACMV		EACMCV	
		S	NS	S	NS	S	NS
Edo	Cassava	20	5	3	1	0	0
	Weed	18	0	1	0	0	0
Ondo	Cassava	6	0	0	0	0	0
	Weed	10	0	1	0	0	0
Anambra	Cassava	6	0	0	0	0	0
	Weed	2	0	0	0	0	0
Delta	Cassava	3	2	0	0	0	0
	Weed	0	0	0	0	0	0
Total		65	7	5	1	0	0

S, Symptomatic; NS, Non symptomatic.

may be indicative of the occurrence of other not tested for in this study and requires further investigation.

CMD remains a threat to cassava production in several African countries. The chances of emergence of new strains/variants of the causative are also very high due to the frequent reports of dual/multiple infections (Fondong et al., 2000; Harrison et al., 1997). Constant surveillance, phytosanitation, and improvement on existing diagnostics will continue to play major roles in ensuring that Nigeria retains its non-epidemic status and low epidemic index (Legg and Owor, 2003). It is also important to ensure that existing quarantine measures

continue to be enforced.

ACKNOWLEDGEMENTS

We thank Dr. S. I. Smith and Mrs. Fowora of the Molecular and Biotechnology Department of the Nigerian Institute of Medical research for bench space, laboratory equipment and assistance to carry out this work.

REFERENCES

Alabi OJ, FO Ogbe, Bandyopadhyay R, Kumar PL, Dixon AGO, d'A Hughes J, Naidu RA (2008a). Alternate hosts of African cassava mosaic virus and East African cassava mosaic Cameroon virus in Nigeria. Arch. Virol. 153(9):1743-1747.

Alabi OJ, Kumar PL, Rayapati AN (2008b). Multiplex PCR for the detection of African cassava mosaic virus and East African cassava mosaic Cameroon virus in cassava. J. Virol. Methods 154:111-120.

Ariyo OA, Koerbler M, Dixon AGO, Atiri GI, Winter S (2005). Molecular Variability and Distribution of Cassava Mosaic Begomoviruses in Nigeria. J. Phytopathol. 153(4):226-231(6).

Dellaporta SL, Wood JJ, Hicks JB (1983). A plant DNA minipreparation: version II. Plant Mol. Biol. Rep. 1:19-21.

FAO (2008). Food and Agricultural Organization of the United Nations. Production Yearbook. FAO Statistics 2007. Rome, Italy: http://faostat.fao.org/.

Fauquet CM, Briddon RW, Brown JK, Moriones E, Stanley J, Zerbini M, Zhou X (2008). Geminivirus strain demarcation and nomenclature. Arch. Virol. 153:783-821.

Fauquet CM, Stanley J (2003). Geminivirus classification and nomenclature: progress and problems. Ann. Appl. Biol. 142:165-189.

Fondong V, Pita JS, Rey MEC, de Kochko A, Beachy RN, Fauquet CM (2000). Evidence of synergism between African cassava mosaic virus and the new double recombinant geminivirus infecting cassava in Cameroon. J. General Virol. 81:287-297.

Harrison BD, Zhou X, Otim-Nape GW, Liu Y, Robinson DJ (1997). Role of a novel type of double infection in the geminivirus-induced epidemic of severe cassava mosaic in Uganda. Ann. Appl. Biol. 131:437-448.

Legg JP, Fauquet CM (2004). Cassava mosaic geminiviruses in Africa. Plant Mol. Biol. 56:585-599.

Legg JP, Owor B (2003). Cassava mosaic disease in Africa: where are the epidemics? Afr. Crop Sci. Conf. Proc. 6:322-328.

Legg JP, Thresh JM (2003). Cassava virus diseases in Africa. In: Proceedings of the First International Conference on Plant Virology in Sub-Saharan Africa (4–8 June 2001, Ibadan, Nigeria), IITA, Ibadan, Nigeria. pp. 517-522.

Manyong VM, Dixon AGO, Makinde KO, Bokanga M, Whyte J (2000). Impact of IITA-improved germplasm on cassava production in sub-Saharan Africa. International Institute of Tropical Agriculture (IITA), Ibadan. Nigeria. P. 11.

Monde G, Walangululu J, Winter S, Bragard C (2010). Dual infection by cassava begomoviruses in two leguminous species (Fabaceae) in Yangambi, Northeastern Democratic Republic of Congo. Arch. Virol. 155(11):1865-1869.

Nassar NMA, Ortiz R (2007). Cassava improvement: challenges and impacts. J. Agric. Sci. 145:163-171.

Ogbe FO, Atiri GI, Dixon AGO, Thottappilly G (2003b). Cassava mosaic disease and its causal agents: the Nigerian situation In: Proceedings of the First International Conference on Plant Virology in Sub-Saharan Africa (4–8 June 2001, Ibadan, Nigeria), IITA, Ibadan, Nigeria. pp. 411-422.

Ogbe FO, Dixon AGO, d'A HughesJ, Alabi OJ, Okechukwu R (2006). Status of cassava begomoviruses and their new natural hosts in Nigeria. Plant Dis. 90:548-553.

Ogbe FO, Thottappilly G, Dixon AGO, Atiri GI, Mignouna HD (2003a). Variants of East African cassava mosaic virus and its distribution in double infections with African cassava mosaic virus in Nigeria. Plant Dis. 87:229-232.

O'Hair SK (1990). Tropical Root and Tuber Crops. In: Janick J, JE Simon (eds.) Advances in new crops. Timber Press, Portland. Oregon. pp. 424-428.

Thresh JM, Otim-Nape GW, Legg JP, Fargette D (1997). African cassava mosaic disease: The magnitude of the problem. In: Contributions of Biotechnology to Cassava for Africa: Proceedings of the Cassava Biotechnology Network, Third International Scientific Meeting (Kampala, Uganda, 26–31 August 1996). A.M. Thro and M.O. Akoroda (Eds.). Afr. J. Root Tuber Crops (Special Issue). pp. 13-19.

Thresh JM, Cooter RJ (2005). Strategies for controlling cassava mosaic virus disease in Africa. Plant Pathol. 54:587-614.