Full Length Research Paper

Shoot nodal culture and virus indexing of selected local and improved genotypes of cassava (*Manihot esculenta*) from Sierra Leone

Janatu V. Sesay[1], Kwadwo O. Ayeh[2], Prince E. Norman[1]* and E. Acheampong[2]

[1]Germplasm Enhancement and Seeds Systems, Njala Agricultural Research Centre (NARC), P. M. B 540, Freetown, Sierra Leone.
[2]Department of Botany, University of Ghana, Box LG 55, Legon, Accra, Ghana.

Cassava (*Manihotes culenta* Crantz) is among major food and income security crops in sub-Saharan Africa. However, high seed dormancy and delayed germination limit seed propagation. Using traditional stem cutting causes loss of superior genotypes and decreases productivity as a result of low multiplication ratio (1:10) and viral and bacterial diseases.Thus, the aim of this study was to optimize and screen efficient in vitro protocols for rapid multiplication and production of disease-free cassava planting materials through nodal culture technique. The experiment was laid out in a 5 x 5 factorial arrangement in Completely Randomized Design with five cassava genotypes (Warima, Munafa, SLICASS 6, Coco cassada and SLICASS 7) and five BAP supplemented MS medium (0, 0.02, 0.1, 1.0 and 2.0 mg/L), replicated three times. Results revealed that, BAP supplement significantly (p<0.001) influenced numbers of leaves and shoots compared to plantlet height growth traits of nodal cassava explants in culture. Virus indexing of infected plants from screen house using species specific primer pairs, OjaRep/EACVMRep and OjaRep/ACMVRep, confirmed the presence of East Africa Cassava Mosaic Virus (EACMV) and the African Cassava Mosaic Virus (ACMV) at an amplicon of approximately 650bp and 400bp, respectively. The study demonstrated the effectiveness of cytokinin supplemented MS medium in enhancing growth of cassava; and the adequacy and effectiveness of the PCR technique in identifying ACMV and EACMV using nodal cuttings. Future studies will involve molecular characterization of the EACMV strain(s) existing in the country.

Key words: Cassava, nodal culture, virus indexing.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a major food security crop providing about 500 calories per day for 800 million people in sub-Saharan Africa and other regions of the world (Nassar et al., 2009). It is the third most

*Corresponding author. E-mail: p.norman@slari.gov.sl. Tel +232 76 410 152.

important source of calories, after rice and maize for many populations in the humid tropics (FAO, 2015). Currently, cassava is cultivated by millions of small-scale farmers in more than 100 countries in the world (FAO, 2013). It is widely grown by farmers because of its remarkable characteristics such as reliability and cheap source of available year-round food, reasonable yields on marginal soils, tolerant to major pests, diseases and drought (Dawit, 2010).

The global production of cassava was estimated at 152 MT per year, of which, 50% was cultivated by small scale farmers in Africa, 30% in Asia and 20% in America (CIAT, 2001; FAO, 2013). Sub-Saharan Africa is major contributor to the global trends in the development of the cassava value chain. However, new uses of cassava are emerging as commercial livestock feed, partial substitute for wheat flour in bread making; and as an industrial raw material such as starch (FAO, 2013). The role of cassava as a traditional food for human consumption is rapidly changing to that of an efficient industrial crop in some parts of Africa including Sierra Leone, where it is considered as the second most important staple food (Nweke, 2004; NARCC/MAFFS, 2005). The crop plays a significant role in the drive to national economic recovery in Sierra Leone with potential spill over effects to neighbouring countries (FAO, 2008). As a major carbohydrate crop, it is a versatile resource with potential for creating diverse products such as chips, broken dried roots meal, starch, flour and ethanol (Kuiper et al., 2007; Heuzé et al., 2015). Dried cassava roots and meal are used as raw material for animal feed, while cassava starch is used for industrial purposes (NARCC/MAFFS, 2005).

Despite its importance, the traditional stem cutting method used for its propagation is one of the bottlenecks for production of disease free planting materials (Ogero et al., 2012). Cassava seeds are normally dormant and germinate very slowly; consequently, farmers utilize the conventional stem cutting propagation method. This method leads to viral, fungal and bacterial diseases which may contribute to decreasing cassava productivity and loss of superior genotypes (Nassar and Ortiz, 2007). The cassava mosaic disease alone has been reported to account for an overall loss of 15-24% in Africa, which is equivalent to 12-23 MT of cassava (Acheremu, 2011). Furthermore, Zhang et al. (2005) noted a yield loss of 19.6 to 27.8% of the actual cassava production in Africa.

Viral diseases lack effective chemical control strategy compared to bacterial and fungal diseases (Jones et al., 2012). Severely infected plants incur heavy yield losses of over 25%, which necessitate the development and use of efficient micro-propagation protocols for healthy planting materials (Acheremu, 2011). However, thermotherapy and chemotherapy techniques can also be used for production of healthy planting materials (Panattoni et al., 2013).

Plant tissue culture is used for the growth and multiplication of cells, tissues and organs under an aseptic and controlled environment, and for rapid clonal propagation culture, somatic embryogenesis, embryo rescue and germplasm exchange (Rao, 1996). The process depends on the fact that cells are totipotent, with each cell capable of expressing the full genome and reproducing the whole plant (Loyola-Vargas and Vázques-Flota, 2006). Micro-propagation techniques are used for rapid clonal multiplication of selected genotypes of diverse plant species (Rani and Raina, 2000).

In vitro culture has contributed significantly to crop improvement by overcoming certain limitations associated with conventional techniques (Garcia-Gonzales et al., 2010). Culturing of an organized tissue in the form of very small shoots or meristem has allowed the most valuable application of plant tissue culture in order to eliminate virus from infected mother plant (Dawit, 2010). For instance, meristem-tip culture has been widely used to remove yield-limiting pathogens from plant cuttings (Acheremu, 2011). The major merits of tissue culture include the elimination of pathogens in donor plants and maintenance of stability in genetic inheritance (Dawit, 2010). The full potential of cassava will not be realized until production constraints are reduced in high-yielding varieties and cassava producers have access to disease-free planting materials. Therefore, the aim of this study was to optimize and screen efficient in vitro protocols for rapid multiplication and production of disease-free cassava planting materials through tissue culture techniques.

MATERIALS AND METHODS

Plant material

The study was conducted at the Botany Department of the University of Ghana, Ghana. A total of five cassava genotypes consisting of two improved: SLICASS 6 and SLICASS 7 and three local: Warima, Munafa and Coco cassada exhibiting different reactions to diseases in Sierra Leone were used in this study. Prior to planting, healthy cuttings each 10 cm long, were obtained from the middle portion of one year old cassava stems. They were pre-treated by soaking in 0.5 g/L Goldazim systemic fungicide in order to protect them from fungal attack and planted in polythene bags filled with well sieved rich top-soil spaced 0.25 m apart on the 9th of September 2014. Each bag was fertigated weekly at the rate of 15 g/L of NPK (20:20:20) throughout the growing period and hand weeded when necessary.

Collection, sterilization and initiation of explants

Nodal cuttings of each genotype measuring 1 cm long each were excised from six weeks old plants in the screen house. The explants were washed twice under clean running tap water and rinsed once with autoclaved water. The explants were surface sterilized by immersing in 70% ethanol for 3 min, and placed in a solution containing 15% sodium hypochlorite and two drops of

Table 1. Primers for PCR amplification and strain identification of cassava mosaic diseases.

Virus strain	Name of primer	Primer sequence	References
ACMV	OjaRep-F ACMVRep-R	CRTCAATGACGTTGTACCA CAGCGGMAGTAAGTCMGA	Alabi et al. (2008)
EACMV	OjaRep-F EACMVRep-R	CRTCAATGACGTTGTACCA GGTTTGCAGAGAACTACATC	Alabi et al. (2008)

tween 80 and kept for 15 min in the lamina flow hood cabinet. The edges of the explants were trimmed and surface sterilized in 10% sodium hypochlorite for 10 min and rinsed with sterile water. The explants were inoculated in 10 mL of each growth medium, covered and then sealed with parafilm and kept in the incubation room.

Nodal explants were initiated in test tubes containing MS medium supplemented with 30 g sucrose, 100 mg/L myo-inositol, 8500 mg/L agar, 1 mg/L thiamine, 1.6 mg/L GA$_3$ and 0.01 g/L NAA at 5.8% pH. The cultures were incubated in an air conditioned room at a temperature of 25±1°C, light intensity of 4000 lux provided by fluorescent bulbs and photoperiod of 16 h light and 8 h dark cycles. Sub-culturing was done at an interval of every four weeks.

Acclimatization of plantlets in the screen house

After root initiation within four weeks, plantlets were carefully removed from test tubes and washed in distilled water to remove the MS medium from the roots. The roots were dipped into antifungal redomil solution and transferred into large plastic bag in a screen-house to minimize shock from the environment. The plastic bag was gradually punched every three days to allow gentle aeration and acclimatization of tissue-cultured plants. The plants were finally weaned after six weeks and transplanted into 2 L volume plastic pots in the nursery.

Treatments, experimental design, phenotypic data collection and analysis

The treatments consisted of five varieties (Warima, Munafa, Coco cassada, SLICASS 6 and SLICASS 7) and five levels of BAP (0, 0.02, 0.10, 1.00 and 2.00 mg/L). The BAP supplemented MS medium trial was laid out in a 5 x 5 factorial arrangement in Completely Randomized Design (CRD) with three replications. Phenotypic data collected were: number of leaves, number of shoots and height of BAP supplemented plantlets.

Data were subjected to analysis of variance (ANOVA) using the GENSTAT statistical program (GENSTAT, 15th release, Rothampstead, UK). The Least Significance Difference (LSD) was used to compare between treatment means using a significance level of α = 0.05.

Extraction of genomic DNA

Genomic cassava DNA was extracted from leaves of tissue-cultured plantlets and those of uncultured sprouted shoot of explant exhibiting various degrees of severity symptoms. The DNA samples were extracted using a slightly modified cetyltrimethylammonium bromide (CTAB) method by Aldrich and Cullis (1993). About 50 mg each of fresh young healthy and diseased leaf samples were grinded separately in liquid nitrogen to a fine powder with 500 µl of 2% CTAB and 0.1% (0.5 µl) of mercaptoethanol. The content was intermittently vortexed in 1.5 ml Eppendorf tube. This was followed by addition of 300 µl of 2% CTAB and 0.5 µl (0.1 %) of ϐ-mercaptoethanol and incubated in a water bath at 65°C for 60 min. The contents were cooled at room temperature, and equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed by inversion of the tube to wash DNA from debris. Samples were then centrifuged at 10,000 rpm for 5 min and the supernatant phase of the sample was transferred into a clean 1.5 mL tube. The chloroform: isoamyl alcohol cleaning step was repeated. The precipitation of DNA was done by addition of 200 µl of ice cold isopropanol to the supernatant, kept on ice for 30 min and centrifuged at 10,000 rpm for 5 min. The isopropanol was decanted and the DNA pellet washed with 350 µl washing buffer (70% ethanol) for 15 min and again centrifuged at 6000 rpm for 4 min. The ethanol was decanted and DNA was dried in a vacuum for 60 min and suspended in 100 µl TE buffer (1 mM Tris HCl, pH 8, 0.1 mM EDTA, pH 8) and stored at -20°C. About 10 µl DNA sample and 5 µl sample buffer (Bromophenol blue) from each extract was run simultaneously with λ Hind III molecular marker on 0.8% Agarose gel containing Ethidium bromide.

Preparation of agarose gel and loading of DNA into wells

A 1.5% agarose gel was prepared by dissolving 3.0 g agarose gel (Sigma company) in 100 ml of TAE (242 g Tris base, 57.1 mL glacial acetic, 100 mL 0.5 M EDTA) buffer. The agarose gel was melted in a microwave oven and cooled to 65°C by constant stirring. After cooling, 4 µl of ethidium bromide was added to 100 ml agar and poured into gel plate with comb already set for loading of sample. The agarose gel was allowed to solidify for about 30 min before removing comb and immersing gel into the electrophoresis tank containing 1xTAE buffer. A 2 µl loading buffer was added to 10 µl of each DNA sample and loaded into the wells. The first well was loaded with 10 µl of 100 bp ladder and run at 100 volts for 1.5 h. After electrophoresis, the gel was visualized on a UV light transilluminator and photographed.

Virus indexing using Polymerase Chain Reaction (PCR) method

The ACMV and EACMV strains causing the mosaic symptoms in leaves of cassava genotypes grown in screen house and *in vitro* culture were detected using PCR method described by Fondong et al. (2000). The DNA samples of the cassava genotypes were tested for presence or absence of CMD using specific primers that could detect the strains of ACMV and EACMV. Two pairs of primer sequences designed by Mills et al. (1992) were used (Table 1).

The PCR analysis of the DNA of each of the 10 samples was performed in a BIO-RAD PTC-220 thermocycler (Dyad MJ Research) for 30 cycles of 35 s at 94°C, 35 s at 55°C, and 1 min at 72°C, and an additional 10 min cycle at 72°C. Reactions were carried out in a total volume of 25 µL containing 0.2 µM dNTPs, 0.5

Table 2. The effect of BAP concentrations on mean number of leaves developed from nodal cuttings at 4 weeks after establishment.

Genotypes	BAP concentration (mg/L)				
	0.0	0.02	0.1	1.0	2.0
Warima	5.3	13.0	11.7	14.0	17.7
Munafa	3.0	11.3	12.3	12.3	5.7
SLICASS 6	22.7	10.0	19.7	19.7	5.0
Coco cassada	3.0	17.0	15.3	15.3	8.7
SLICASS 7	3.7	1.0	2.0	2.0	5.7
Mean	7.5	10.5	12.2	12.7	8.5
$LSD_{(0.05)gen}$	3.1***				
$LSD_{(0.05)conc}$	3.1**				
$LSD_{(0.05)genxconc}$	7.0***				

Gen=genotype, conc=concentration, **= significant at $p<0.01$ and ***=significant at $p<0.001$.

Figure 1. Leaves of plantlets developed from nodal cuttings at 4 weeks after establishment on BAP culture. a=Warima b=Munafa, c=SLICASS 6, d=Coco cassada, e=SLICASS 7.

µM primers, 2.5 µM 1x PCR amplification buffer, 0.5 mM $MgCl_2$, 0.4U Taq DNA polymerase (Invitrogen), 0.4 µM DNA template and 50 ng ultrapure water. Reactions were done twice to evaluate the consistency of the banding patterns for all isolates studied. Products were separated on a 2% (w/v) agarose gel in 1x TAE buffer (40 mMTris acetate, 1 mM EDTA, pH 8.0) at 125 V for 3 h. The gels were stained with ethidium bromide and visualized with UV light using a Cole Palmer FLUO-LINK FLX apparatus and photographed for later assessment.

RESULTS

Generally, the number of leaves per plantlet significantly ($p<0.01$) varied with genotype, different levels of BAP and interaction between both (Table 1). Genotype SLICASS 6 (22.7) produced the highest number of leaves at 0 mg/L BAP compared to the remaining genotypes which increased with varying levels of BAP (Table 2, Figure 1). Genotypes Warima (17.7) and SLICASS 7 (5.7) produced highest number of leaves at 2.0 mg/L BAP; whereas, the highest number of leaves in Munafa (12.3) and Coco cassada (17.0) were at 0.1 and 0.02 mg/L BAP, respectively. The results indicated that profuse leaf production depends on the genotype, growing medium and interaction within the growing environment.

The number of shoots developed from nodal cuttings at 4 weeks after establishment significantly ($p<0.001$) differed with genotypes, different levels of BAP and interaction between both (Table 3). Generally, BAP induced multiple shoot development from buds in all cassava genotypes. Mean number of shoots ranges from 1 (SLICASS 7) to 10 (SLICASS 6). The highest number of shoots produced in Warima and Munafa on 0.1 mg/L BAP were 6 and 10, respectively; whereas, the highest for SLICASS 6 (10.3), Coco cassada (8.7) and SLICASS 7 (5.0) were at 1.0, 0.02 and 2.0 mg/L BAP, respectively. These implied that, no single BAP concentration is optimum for mean number of shoot production in the five cassava genotypes studied; and that each genotype

Table 3. Effect of BAP on number of shoots developed from nodal cuttings at 4 weeks after establishment.

Genotypes	BAP concentration (mg/L)				
	0.0	0.02	0.1	1.0	2.0
Warima	3.3	5.0	6.0	5.3	4.3
Munafa	3.7	5.7	9.7	5.3	3.3
SLICASS 6	4.7	7.0	7.7	10.3	3.0
Coco cassada	3.3	8.7	8.0	0.7	3.7
SLICASS 7	3.0	0.7	3.3	2.7	5.0
Mean	3.6	5.4	6.9	4.9	3.9
LSD$_{(0.05)gen}$	1.4***				
LSD$_{(0.05)conc}$	1.4***				
LSD$_{(0.05)genxconc}$	3.2***				

Gen=Genotype, conc=Concentration and ***= Significant at $p<0.001$.

Figure 2. Mean height (cm) of plantlets developed from nodal cuttings at 4 weeks after establishment on BAP culture. a=Warima b=Munafa, c=SLICASS 6, d=Coco cassada, e=SLICASS 7.

produces the highest mean number of shoot in a medium supplemented with a specific concentration of BAP.

The effect of various BAP amended culture media on the mean plantlet height is presented in Table 4 and Figure 2. Addition of BAP at 0.02, 0.1 and 1.0 mg/L induced taller plantlets compared to 2.0 mg/L BAP concentration. The tallest mean plantlet height (3.7 cm) was obtained in SLICASS 6 amended at 0.1 mg/L BAP, followed by SLICASS 7 (3.2 cm) and Coco cassada (2.6 cm) amended at 0.1 mg/L, whereas Warima (0.8 cm) had the shortest mean plantlet height at 0.0 mg/L (Table 4). These findings implied that, cassava genotypes responded differently to various concentrations of BAP; thus, genotype Warima was consistently among genotypes that exhibited shortest plantlets across the various BAP media.

Virus indexing

Amplification of the EACMV with the species specific primer pair (OjaRep/EACVMRep) did not generate any

PCR products at 650 bp for healthy cassava plantlets from the growth room (lanes 1, 3 and 4), whereas lanes 6, 8 and 10 from the screen house and lanes 2 and 5 from the growth room were +ve for EACMV (Figure 3). Unlike the negative control (-ve), which did not produce PCR product, the positive control (+ve) (infected plants) produced an amplicon at approximately 650 bp (Figure 3). Similar trends were observed for OjaRep/ACMVRep specific primers in which the positive control (+ve) and lanes 2, 5, 6, 8 and 10 exhibited an amplicon at approximately 400 bp (Figure 4).

DISCUSSION

Findings in this study showed that genotype SLICASS 6 developed highest number of leaves in 0 mg/L BAP, whereas genotypes Warima, Munafa, Coco cassada and SLICASS 7 required higher BAP supplement to produce the highest mean number of leaves. This implies that different genotypes probably possess different levels of endogenous hormones needed as amendment of the MS

Table 4. Effect of BAP on cassava plantlet height (cm) developed from nodal cuttings at 4 weeks after establishment.

Genotypes	BAP concentration (mg/L)				
	0.0	0.02	0.1	1.0	2.0
Warima	0.8	1.7	1.3	1.1	1.3
Munafa	1.6	2.5	2.3	1.5	1.9
SLICASS 6	3.0	2.9	3.7	3.4	1.4
Coco cassada	1.9	1.7	2.6	2.4	2.6
SLICASS 7	2.5	2.5	3.2	2.4	1.0
Mean	2.0	2.3	2.6	2.2	1.7
$LSD_{(0.05)gen}$	0.8**				
$LSD_{(0.05)conc}$	0.8^{ns}				
$LSD_{(0.05)genxconc}$	1.8^{ns}				

Gen=genotype, conc=concentration, ns and **=not significant and significant at $p<0.01$, respectively.
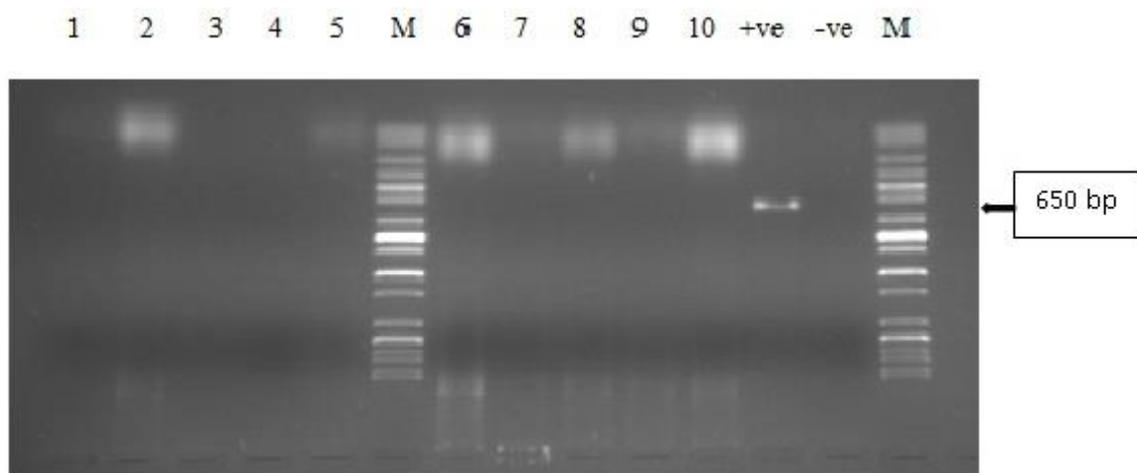

Figure 3. Virus indexing of EACMV using PCR: Amplified DNA fragments of cassava plantlets in growth room (lanes 1 to 5) and screen house (lanes 6 to 10) using OjaRep/ACMVRep species specific primer pair. M = Molecular size marker GeneRuler™ DNA Ladder Mix (100-10,000 bp); +ve = Positive control (ACMV reference sample); -ve = Negative control (sterile distilled water).

medium and that their concentrations in some genotypes may either be sub or supra optimal for leaf production. The present finding agrees with Escobar et al. (1999) who reported that the response among varieties was primarily due to the presence of various levels of cytokinin in explants. Similarly, Acedo and Labana (2008) noted rapid propagation of released Philippine cassava varieties through tissue culture, suggesting their genotypic specificity to culture medium. Moreover, the leaves produced from SLICASS 6 were morphologically more vigorous and had deep green colour than all other genotypes. The variation in the colour of the regenerated leaves might be due to the endogenous cytokinin which enhanced chlorophyll development in the leaves of SLICASS 6 as compared to the leaves of the others. This might suggest that, chlorophyll development in the five cassava genotypes are possibly controlled by different genes. The observation made by Konan and co-workers showed different responses of number of leaves generated by different cassava varieties in-vitro (Konan et al., 2006).

Cassava exhibits strong apical dominance, both in vivo and in-vitro cultures. Breaking of the apical dominance results in multiple shoot production. In this study, multiple shoots produced by genotypes SLICASS 6, Coco cassada, Warima, Munafa and SLICASS 7 from the single nodal cutting cultures were possibly due to the BAP concentration of the culture medium. These findings agree with Demeske et al. (2014) who reported an average of 7.3 shoots per explants at 0.75 mg/L BAP
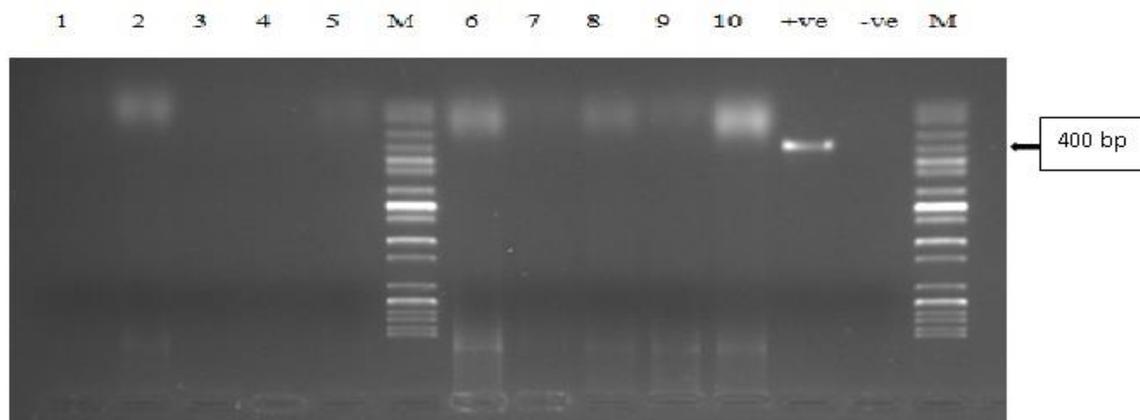
Figure 4. Virus indexing of ACMV using PCR: Amplified DNA fragments of cassava plantlets in growth room (lanes 1 to 5) and screenhouse (lanes 6 to 10) using OjaRep/ACMVRep species specific primer pair. M = Molecular size marker GeneRuler™ DNA Ladder Mix (100-10,000 bp); +ve = Positive control (ACMV reference sample); -ve = Negative control (sterile distilled water).

compared to other media concentrations. Similarly, earlier researchers noted multiple shoots from nodal cuttings of cassava on a medium supplemented with 1.0 mg/L BAP (Smith et al., 1986). Their findings revealed that about 20 shoots were harvested every three weeks, but later decreased as time progressed, from the rosette multiple shoot culture during the multiplication stage. The results in this study confirmed that, multiple shoot production is dependent on the BAP concentration in the medium as well as the cassava genotypes.

Results on plantlet height agree with Mushiyimana et al. (2011) who observed that culturing nodal explants harvested from *in vitro* grown plantlets on different MS formulations supplemented with 5, 10 20 and 40 µM/L GA$_3$ produce significant shoot elongation in cassava. Mushiyimana et al. (2011) suggested that, the best regeneration medium for cassava explants is full strength MS medium supplemented with 40 µM/L GA$_3$ provided that, the highest mean shoot length of 8.93±2.67 mm. Acedo (2006) worked on improvement of *in vitro* techniques for rapid meristem development and mass propagation of Philippine cassava using both liquid and solid MS media supplemented with GA$_3$, BAP and NAA. The present study partly agree with his report that plant regeneration was most effectively promoted by liquid MS medium supplemented with GA$_3$, BAP and NAA produced 2-nodal stages complete plantlet with callus after three weeks from inoculation. However, when the hormones were used singly, GA$_3$ did not show growth response whereas BAP caused shoot formation.

BAP is the most widely used phytohormone/cytokinin and it is effective at 0.01 – 10 mg/L for plantlet height. However, all the plantlet heights considered were significantly different at various concentrations. The interaction between BAP and genotype was also significantly (p<0.01) different on plantlet leaves and shoots evaluated. This could be associated with the effectiveness of cytokinin even at low concentration in cassava which was consistent with previous findings (Onuoch and Onwubiku, 2007; Staden et al., 2008).

Viral indexing

The success of virus indexing depends on an effective method of virus detection. A good test is needed to ensure that, plantlets regenerated from meristem culture are disease-free (Milošević et al., 2012). The DNA extracted from infected cassava leaves was used as positive control and it revealed that cassava genotypes studied were infected with both ACMV and EACMV diseases. The DNA of virus indexed genotypes in lanes 2, 5, 6, 8 and 10 exhibited infection of the diseases possibly due to contamination of samples. The DNA of the untreated positive control exhibited bands on the gel electrophoresis visualized, implying the presence of disease on infected cassava leaves that had not been virus indexed (Figures 6 and 7). The PCR results of treated or virus indexed plantlets from growth room (non-acclimatized) and screen-house (acclimatized) confirmed that, some plants were virus-free. Sseruwagi et al. (2004) noted known occurrence of ACMV in most cassava growing regions of Africa including Sierra Leone, whereas the EACMV has not widely spread (Neuenschwander et al., 2002; Freddy et al., 2015). The discovery of EACMV in genotypes of cassava from Sierra Leone is the first report of the disease. However, the strain(s) in the country are yet to be investigated. The

PCR technique used in this study distinguished between virus infected and non-infected plants similar to those noted by (Ogbe et al., 2003).

Conclusion

This study showed that growth architecture of cassava depends on genotype, growing medium and interaction of growing environment with genotype. The cytokinin, BAP, was effective in enhancing growth of cassava. The nodal culture technique applied was adequate and effective in eliminating viruses in some genotypes from Sierra Leone. The PCR technique confirmed the presence of ACMV and EACMV in some cassava indicating the need for thorough and routine cleaning of cassava planting materials against viruses. Future studies will target the molecular characterization of different strains of viruses existing within cassava germplasm in the country, multiplication and/or conservation of virus indexed or virus free materials for utilization in breeding and crop production.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

REFERENCES

Acedo VZ (2006). Improvement of *in vitro* techniques for rapid meristem development and mass propagation of Philippine cassava (*Manihot esculenta* Crantz). J. Food Agric. Environ. 4(1):220-224.

Acedo VZ, Labana CU (2008). Rapid propagation of released Philippine cassava varieties through tissue culture. J. Root Crops 34(2):108-144.

Acheremu K (2011). Use of thermotherapy and tissue culture to free diseased cassava cultivars from cassava mosaic disease. Thesis, College of Agric. and Natural Resources.

Alabi OJ, Kumar PL, Naidu RA (2008). Multiplex PCR method for the detection of Africa Cassava Mosaic virus and East Africa Cassava Mosaic Virus in Cassava. J. Viral Met. 154:111-120.

Aldrich J, Cullis CA (1993). RAPD analysis in flax: Optimization of yield and reproducibility using klenTaq 1 DNA polymerase, chelex 100, and gel purification of genomic DNA. Plant Mol. Biol. Report. 11(2):128-141.

CIAT (Centro Internacional de Agricultura Tropical) (2001). Project IP3, Improved cassava for the developing World. Annual Report 2001, ApdoAereo 6713, Cali, Colombia.

Dawit B (2010). Micropropagation of selected cassava varieties (*Manihot esculenta* Crantz) from meristem culture. MSc thesis department of Biology, Addis Ababa University, Ethiopia. 46 p.

Escobar R, Munoz L, Roca, WM (2009). Cassava Micropropagation for

Rapid "Seed Production" Using Temporary Immersion Bioreactors. International Center of Tropical Agriculture (CIAT). Cali, Columbia. [Online] Available from: http://www.redbio.Org/portal/encounters/enc2001/posters/07/pdf/Sin%20registro5.pdf. [Accessed on 21 January, 2010].

FAO (2015). Regional Conference on Cassava In the Caribbean and Latin America, Conference Report 10-12 February 2014. 99 p.

FAO (2013). FAOSTAT statistical database (http://faostat.fao.org).

FAO (2008). Cassava as alternative food source. Food and Agriculture Organization of the United Nations, Rome (http://www.bruneitimes.com.bn) (Accessed on June 21, 2009).

Fondong VN, Pita JS, Rey MEC, De Kochko A, Beachy RN, Fauquet CM (2000). Evidence of synergism between African cassava mosaic virus and a new double combination virus infecting cassava in Cameroon. J. Gen.Virol. 81:287-297.

Freddy BO, Diamuini NA, Kikakedimau NR, Mbaya NA, Mutambel H, Lumande K, Luyindula N, Rufflard G, Lepoivre P (2015). PCR and ELISA detection of cassava mosaic virus in a Congolese cassava landrace. Int. J. Biotechnol. Food Sci. 3(1):10-16.

Heuzé V, Tran G, Archimède H, Régnier C, Bastianelli D, Lebas F (2015). *Cassava roots*. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. http://www.feedipedia.org/node/527

Garcia-Gonzales R, Quiroz K, Carrasco B, Caligari P (2010). Plant tissue culture: Current status, opportunities and challenges. Cien. Inv. Agric. 37(3):5-30.

Jones JB, Vallad GE, Iriarte FB, Obradović, A, Wernsing MH, Jackson LE, Balogh B, Hong JC, Timur Momol M (2012). Considerations for using bacteriophages for plant disease control Bacteriophage. 2(4):208-214.

Konan NK, Sangwan RS, Sangwan-Norreel BS (2006). Efficient in vitro Shoot-regeneration Systems in Cassava (Manihot esculenta Crantz). Plant Breed. 113(3):227-236.

Kuiper L, Ekmecki B, Hamelink C, Hettinga W, Meyer S, Koop K (2007). Bio-ethanol from cassava. Project number: PBIONL062937. Ecofys Netherlands BV, Utrecht

Ogero, KO, Mburugu GN, Mwangi M, Ombori O, Ngugi M (2012). In vitro Micropropagation of Cassava Through Low Cost Tissue Culture. Asian J. Agric. Sci. 4(3):205-209.

Loyola-Vargas VM, Vázques-Flota F (2006). Plant Cell Culture Protocols. Second Edition. In: Loyola-Vargas, V.M. and Vázquez-Flota, F. eds. Humana Press Inc., New Jersey. Methods Mol. Biol: 318:3-8.

MAFFS/NARCC (Ministry of Agriculture, Forestry and Food Security) / (National Agricultural Research Coordinating Council) (2005). Crop production guidelines for Sierra Leone. 104 p.

Milošević S, Cinge A, Jevremović S, Stanković I, Bulajić A, Krstić B, Subotić A (2012). Virus Elimination from Ornamental Plants Using in vitro Culture Techniques, Pestic. Phytomed. (Belgrade), 27(3):203-211.

Nassar NMA, Junior OP, Sousa MV, Ortiz R (2009). Improving Carotenoids and Amino-Acids in Cassava. Recent Pat. Food Nutr. Agric. 1(1):32-38.

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

Neuenschwander P, Hughes J, Ogbe FO, Ngatse JM, Legg JP (2002). The occurrence of the Uganda Variant of East Africa cassava mosaic virus (EACMV-UG) in western Democratic Republic of Congo and the Congo Republic defines the westernmost extent of the CMD pandemic in East/Central Africa. Pathol. Seed. 51(3):384.

Nweke F (2004). New challenges in the cassava transformation in Nigeria and Ghana. Discussion paper No. 118. Environment and Production Technology Division, International Food Policy Research Institute, IFPRI.

Ogbe FO, Atiri GI, Dixon AGO, Thottappilly G (2003). Symptom severity of cassava mosaic disease in relation to concentration of ACMV in different cassava genotypes. Plant Pathol. 52(1):84-91.

Onuoch CI, Onwubiku NJC (2007). Micropropagation of Cassava (*Manihot esculenta* Crantz) using different consentration of Benzyaminiopurin (BAP). J. Eng. Appl. Sci. 2(7):1229-1231.

Panattoni A, Luvisi A, Triolo E (2013). Review. Elimination of viruses in

plants: twenty years of progress. Span. J. Agric. Res. 11(1):173-188.

Rani V, Raina SN (2000). Genetic fidelity of organized meristem-derived micropropagated plants: A critical reappraisal. *In vitro* Cellular Dev. Biol. Plant 36(5):319-330.

Rao PVL (1996). Plant Biotechnology: Promises and Challenges. Def. Sci. J. 46(1):31-39.

Smith MK, Biggs BJ, Scott KJ (1986). In vitro propagation of cassava (*Manihot esculenta* Crantz). Plant Cell Tissue Organ Cult. 6(3):221-228.

Sseruwagi P, Sserumbombwe WS, Legg JP, Ndunguru J, Thresh JM (2004). Methods for surveying the incidence and severity of cassava mosaic disease and whitefly vector populations on cassava in Africa: A review. Virus Res. 100:129-142.

Staden JV, Zazimalova E, George EF (2008). Plant Growth Regulators II: Cytokinins, their Analogues and Antagonists. In: Plant Propagation by Tissue Culture 3rd ed. (George, E. F., Hall, M.A. and De Klerk, G. eds.) Springer. pp. 205-226.

Zhang P, Vanderschuren H, Fütterer J, Gruissem W (2005). Resistance to cassava mosaic disease in transgenic cassava expressing antisense RNAs targeting virus replication genes. Plant Biotechnol. J. 3(4):385-397.