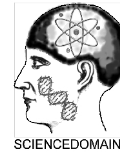
British Biotechnology Journal
3(3): 263-273, 2013

SCIENCEDOMAIN *international*
www.sciencedomain.org



Primary Somatic Embryos from Axillary Meristems and Immature Leaf Lobes of Selected African Cassava Varieties

Jelili T. Opabode[1*], Olufemi O. Oyelakin[2,3], Oluyemisi A. Akinyemiju[1] and Ivan L. Ingelbrecht[2,4]

[1]Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife, Nigeria.
[2]Central Biotechnology Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria; c/o Lambourn Ltd, Carolyn House, 26 Dingwall Road, Croydon CR9 3EE, UK.
[3]Biotechnology Center, Federal University of Agriculture, Abeokuta, Nigeria.
[4]Department of Plant Biotechnology and Bio-informatics, Ghent University, K L Ledeganckstraat 35, B-9000 Ghent, Belgium.

Authors' contributions

This work is a portion of the Ph.D Thesis of the first author JTO. The authors OOO, OAA, and ILI read and approved the final manuscript.

Research Article

Received 19th January 2013
Accepted 1st March 2013
Published 5th April 2013

ABSTRACT

The study evaluated high-value African cassava varieties for primary somatic embryogenesis using *axillary* meristems (AM) and immature leaf lobes (LL) on picloram-based medium. The study was conducted at the Central Biotech Lab, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria between 2006 and 2009. Completely randomized design with four replicates was used for the study. Using LL explants, there were significant (*P=.05*) differences in percent responding leaf lobes, percent explant with pre-embryogenic structure, PSEF and PSEE among cassava varieties. The PSEF of the only three varieties that produced mature somatic embryo were 93.6, 88.5 and 85.7% for TME 12, Kibaha and Albert, respectively. Similarly, significant (*P=.05*) differences existed among the varieties in percent enlarged axillary meristem, percent explant with pre-embryogenic structure, PSEF and PSEE when AM

**Corresponding author: Email: jopabode@yahoo.com;*

was the explant. The PSEFs of the only three varieties that produced mature somatic embryo were 83.6, 77.5 and 72.7% for TME 12, Kibaha and Albert, respectively. The PSEF and PSEE of LL explant were greater than those of AM by an average of 86.1% and 82.7%, respectively. The study concluded that both AM and LL were good explants for production of primary somatic embryo in cassava.

Keywords: Axillary meristem; cassava; immature leaf lobe; primary somatic embryogenesis.

ABBREVIATIONS

AM = Axillary meristem; APM=apical meristem; LL =Immature leaf lobe PSE=primary somatic embryogenesis; BM=Basal medium; PSEF=primary somatic embryogenesis frequency; PSEE = Primary somatic embryogenesis efficiency.

1. INTRODUCTION

Cassava is an important source of energy in the diet of livestock and about 600 million people in tropical and subtropical climates [1]. The increase in cassava cultivation and its important role in food security are enhanced by two factors: the unique biology of the crop and the numerous uses to which its starch and by-products are put [2,3,4]. The starch content of cassava roots ranges from 65 - 91% of its total root dry weight depending on the cultivar [5,6]. The global demand for cassava starch is rising and it is fastly replacing conventional sources of starch like wheat, maize, rice and potato because of its enhanced properties [7,8]. Despite its potentials for achieving food security and economic growth, biotic and abiotic constraints such as diseases, pests, weeds, poor soil fertility and drought are militating against cassava production [9,10,11]. Application of conventional breeding methods for improvement of cassava against the biotic and abiotic constraints has so far recorded limited success [9,12]. Conventional breeding of cassava is challenging due to the highly heterozygous nature of the crop which prevents a backcross scheme. In the field, cassava is typically propagated clonally by stem cuttings. This propagation strategy is ideal for a bio-engineering approach to crop improvement as gene segregation throughout crossing is limited [13]. Successful genetic modification by bio-engineering approach requires establishment of *in vitro* regeneration and transformation systems. To date, the only reported means of incorporating foreign pieces of DNA into cassava genome is via regeneration through somatic embryogenesis [13].

Plant regeneration via somatic embryogenesis has become an integral component of genetic transformation system in cassava [14,15]. Regeneration studies have shown that the frequency and efficiency of somatic embryogenesis are highly genotype-dependent, and not all cassava cultivars are amenable to somatic embryogenesis, regeneration and transformation, therefore, there is a need to optimise the generation of embryogenic structures for each cassava cultivar [16,17,18,19,20,21,22]. As a result, more than 60 African cassava cultivars and other cultivars from South America and Asia have been tested for their somatic embryo-producing ability. For the same reasons, somatic embryos have been induced from various cassava explants including immature leaf lobes [16,17,18,19,20,21,22,23,24], shoot apical meristems [16,20,24], zygotic embryos or floral tissue [23], on several media containing various plant growth regulators.

Induction and maturation of somatic embryo from African cassava cultivars have been mostly obtained from immature leaf lobes and shoot apical meristem explants [16,20]. Limited number of studies reported the use of axillary meristem as explants. Rossin and Rey [24] obtained primary somatic embryo from axillary meristem of eight South African cassava cultivars with primary somatic embryo frequencies and efficiencies ranged from 28 to 83% and 1.5 to 4.0, respectively. Compared with apical meristem or leaf lobe explants, axillary meristems occur in large number on plantlets with the advantage of scaling up experiment as high number of axillary meristem can be obtained from *in vitro* plantlets. High number of explants per replicate or experiment increases the chance of obtaining positive response to somatic embryo induction, regeneration and transformation. In addition, the use of axillary meristem provides opportunity to increase the scope of research by including several factors for investigation which expands the information obtainable from such study. In this communication, eleven African cultivars of diverse agronomical traits were screened for somatic embryo using axillary meristems and immature leaf lobes as explants.

2. MATERIALS AND METHODS

2.1 Plant Materials

Cassava (*Manihot esculenta*) plantlets of eleven cultivars (Table 1) were obtained from *in vitro* germplasm collection of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The plantlets were maintained by regular subculturing at four weeks interval as *in vitro* shoot cultures on basal medium (BM).

2.2 Basal Medium and Culture Conditions

Basal medium (BM) which, consisted of full-strength MS [25] salt (Sigma, USA) along with 0.8% (w/v) agar (Oxoid Ltd, England), 30 g/l sucrose and 2μM $CuSO_4$, was used in all experiments unless otherwise stated. The pH of the medium was adjusted to 5.8 by HCl (1 N) or NaOH (1N) prior to autoclaving at 121ºC for 15 minutes at 1.05 kg cm^{-2} pressure. Growth regulators were filter sterilized through 0.22-μM Millipore filters and added to media after autoclaving. For all experiments, cultures were maintained under 16 h photoperiod with 20 μmol m^{-2} s^{-1} light intensity provided by cool-white fluorescent tubes at 25 ± 2ºC.

2.3 Induction and Maturation of Primary Somatic Embryos from Axillary Meristems

The procedure as described by Rossin and Rey [24] was followed for induction of primary somatic embryo from axillary meristem isolated from axillary bud. Nodal explants (2-3 cm) were excised from three-week old *in vitro* plantlets and incubated on BM containing 10 mg/l BAP in the dark for 7 days for enlargement of axillary buds. Lateral meristems (1-2mm) were isolated from the enlarged axillary buds with sterile surgical blade and forcep with the aid of microscope in a horizontal laminar flow bench. The isolated axillary meristems were incubated in BM supplemented with 10 mg/l of picloram for 17 days for induction of primary somatic embryo. Pre-embryogenic structures were later incubated in dark on BM supplemented with 0.1mg/l BAP for 10 days for maturation.

Table 1. Source, agronomic trait and adapted agro-ecologies of some landraces and improved cassava varieties included in the study

s/n	Varieties	Source	Status	Agronomic traits	Adapted agroecology	Explant previously tested for PSE
1	TMS 1425	IITA, Nigeria	improved	High yielding	West Africa	APM, LL
2	Albert	Tanzania	landrace	Suceptible to BSV	East Africa	nil
3	TMS 98/0505	IITA, Nigeria	improved	High yielding, ACMV resistance	Africa	nil
4	TMS 97/3200	IITA, Nigeria	improved	Low cyanogen, High yielding	Africa	nil
5	TMS 98/0068	IITA, Nigeria	improved	High starch, low cyanogen	Africa	nil
6	TMS 91/02324	IITA, Nigeria	improved	Good canopy, weed smothering	Africa	APM, LL
7	TMS 92B/0068	IITA, Nigeria	improved	Suitable for mixed cropping	Humid Africa	nil
8	TMS 97/4768	IITA, Nigeria	improved	Suitable for mixed cropping	Savanna Africa	nil
9	TMS 98/0510	IITA, Nigeria	improved	High yielding, ACMV resistance	Africa	nil
10	Kibaha	Tanzania	landrace	Susceptible to BSV	East Africa	nil
11	TME 12	IITA, Nigeria	landrace	Good canopy, good yielding	Savanna ecologies	APM, LL

APM= apical meristem LL= immature leaf lobe IITA=International Institute of Tropical Agriculture TMS= Tropical Manihot Series TME=Tropical Manihot esculenta ACMV=African cassava mosaic virus BSV=Brown streak virus

2.4 Induction and Maturation of Primary Somatic Embryo from Immature Leaf Lobes

Primary somatic embryo was induced from immature leaf lobe as previously described by [20]. Immature leaf lobes (1-6 mm) obtained from three-week old *in vitro* plantlets were incubated in the dark on BM supplemented with 10 mg/l picloram for 14 days. Pre-embryogenic structures were later incubated in the dark on BM supplemented with 0.1mg/l BAP for 10 days for maturation.

2.5 Experimental Design and Statistical Analysis

All experiments were arranged in completely randomized design with four replicates in each experiment. Each experiment was repeated three times. Primary somatic embryogenesis frequency was defined as the number of explants that produced somatic embryo and expressed as a percentage. Primary somatic embryogenesis efficiency was the number of somatic embryo produced by an explant and expressed as number of somatic embryo per explant. Observation on number of explants with enlarged meristem was made seven days after incubation. Data on the presence of pre-embryogenic structure and matured embryo were collected on 14 and 21 days after incubation, respectively. Data were subjected to arcsine and square root transformations to normalize variances. Data were further subjected to analysis of variance to detect differences among treatments at $P \leq 0.05$ by Duncan's multiple tests as outlined by [26].

3. RESULTS

3.1 Induction and Maturation of Primary Somatic Embryo from Axillary Meristems

Induction and formation of primary somatic embryo via axillary meristem are shown in Fig. 1. Stem segments, each with an axillary node (A), were obtained from three-week old *in vitro* propagated plantlet. Buds break and elongation were found on the axillary node when cultured for 7 days in the dark on BM containing 10mg/l BAP. The auxillary node on A was successfully enlarged with BAP after seven days of dark incubation. B was an isolated meristem from the enlarged node in A which was used to generate embryogenic callus (C) using picloram. Maturation of C with little quantity of BAP produced D with globular primary somatic embryo which fully matured two weeks later as shown in E. All varieties had enlarged node when cultured on meristem enlargement medium however, significant difference *(P=.05*) existed in the percentage of enlarged nodes among varieties (Table 2). Cassava varieties Albert, TME 12, Kibaha, TMS 4 (2) 1425 and TMS 98/0505 recorded not less than 70% explant with enlarged node. Only four varieties produced pre-embryogenic structure when transferred on induction medium. There was significant difference (*P=.05*) in percentage of explants with pre-embryogenic structure among varieties (Table 2). TME 12 recorded the highest (84.6%) percentage of explants with pre-embryogenic structure while TMS 98/0505 had the lowest (10.3%). Pre-embryogenic structure matured into primary somatic embryo in only three varieties which were Albert, Kibaha and TME 12. Variety TME 12 had the largest percentage of mature primary somatic embryo (83.4%) while Albert recorded the lowest (72.7%). The average number of primary somatic embryo per explant in Kibaha (5.6) was significantly higher (*P=.05*) than Albert (4.5) and TME 12 (4.5). Primary somatic embryos were mostly emerged at the base of explant in Albert, while they emerged from the top of the explant in TME 12 (Table 2). In Kibaha, primary somatic embryos were evenly distributed on explant. Primary somatic embryos produced by Albert were mostly separated in shape while that of Kibaha and TME 12 had fused and conical cotyledon, respectively.

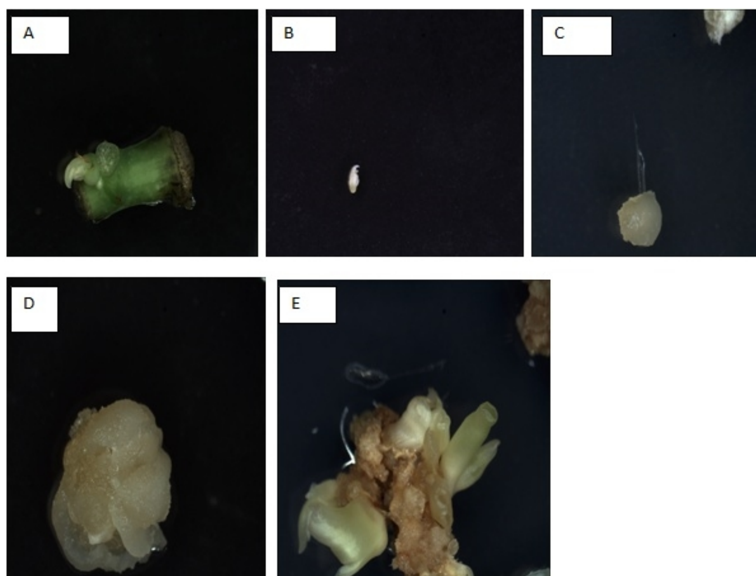


Fig. 1. Production of primary somatic embryo via axillary meristem in cassava variety TME 12. A- stem segment with an enlarged axuxillary node (x1/4), B- isolated shoot meristem (x4), C-2-week old embryogenic callus, D- 4-week old embryogenic callus (x1), E- mature primary somatic embryos (x1)

Table 2. Production of primary somatic embryo using axillary meristem in some selected cassava varieties

variety	% explants with enlarged node	% explants with pre-embryogenic structure	PSEF (%)	PSEF (No/explants)	Location of embryo on explant	Shape of embryo
TMS 1425	75.3±0.8^{b}	0.0^{e}	0.0^{d}	0.0^{c}	-	-
Albert	83.4±1.3^{a}	45.6±0.7^{c}	72.6±3.6^{c}	4.5±1.7^{b}	base	CC
TMS 98/0505	72.6±0.5^{b}	10.3±0.7^{d}	0.0^{d}	0.0^{c}	-	-
TMS 97/3200	60.8±1.5^{d}	0.0^{e}	0.0^{d}	0.0^{c}	-	-
TMS 98/0068	63.7±1.3^{c}	0.0^{e}	0.0^{d}	0.0^{c}	-	-
TMS 91/02324	65.1±3.2^{c}	0.0^{e}	0.0^{d}	0.0^{c}	-	-
TMS 92B/0068	68.3±2.5^{c}	0.0^{e}	0.0^{d}	0.0^{c}	-	-
TMS 97/4768	57.8±0.7^{d}	0.0^{e}	0.0^{d}	0.0^{c}	-	-
TMS 98/0510	54.3±0.8^{d}	0.0^{e}	0.0^{d}	0.0^{c}	-	-
Kibaha	75.4±1.4^{b}	57.4±0.5^{b}	77.6±2.7^{b}	5.6±0.8^{a}	even	FC
TME 12	81.5±1.2^{a}	84.6±2.3^{a}	83.4±4.3^{a}	4.5±0.6^{b}	top	CC
Mean	68.93	17.99	23.98	1.33	-	
CV (%)	19.63	120.01	149.71	2.14	-	

Values are means (±standard error). Values followed by different superscripts in same column are significantly different at P≤0.05 by Duncan's multiple range test. CV= coefficient of variation C=conical cotyledon FC=fused cotyledon

3.2 Induction and Maturation of Primary Somatic Embryo from Immature Leaf Lobes

Induction and maturation of primary somatic embryo from immature leaf lobe in TME 12 is shown in Fig. 2. An immature leaf lobe obtained from two-week old *in vitro* propagated plantlet was decapitated (Fig. 2A). Decapitated leaf lobe inducted for primary somatic embryo with picloram produced Fig. 2B after one week of dark incubation, embryogenic callus after two weeks (Fig. 2C) of dark incubation. Maturation of the embryogenic callus was done with BAP gave D and fully matured primary somatic embryo (E) was successfully obtained after five weeks of dark incubation. The green colour of decapitated immature leaves disappeared as calluses were developing. Callus started developing at 4 days after incubation from the cut edge of the leave and extended into the interior in the responded leaves. Pre-embryogenic structures occupied the central portion of the calluses. The pre-embryogenic structures are globular in shape with bipolar structures and light-brown in colour (Fig.2D). There were significant differences (*P=.05*) in percentages of responded leaf lobe, percent explants with pre-embryogenic structures, frequency and efficiency of primary somatic embryo (Table 3). Variety TME 12 recorded the highest percentage of responded leaf lobe (87.5%) while TMS 1425 had the lowest (62.5%). Only Albert, Kibaha and TME 12 produced pre-embryogenic structures. The percentage of pre-embryogenic structures was highest (84.6%) in TME 12 and lowest (77.4%) in Kibaha. Only three varieties produced mature primary somatic embryo. They were TME 12, Kibaha and Albert with 93.6%, 88.5% and 85.7%, respectively. The efficiency of primary somatic embryo was highest in Kibaha (5.6 embyo/explant). Primary somatic embryos were located at the base of Albert explants, top of TME 12 explants and evenly distributed on the explants of Kibaha. The shapes of primary somatic embryos in the three varieties were: conical in Albert, fused cotyledon in Kibaha and separated cotyledon in TME 12.

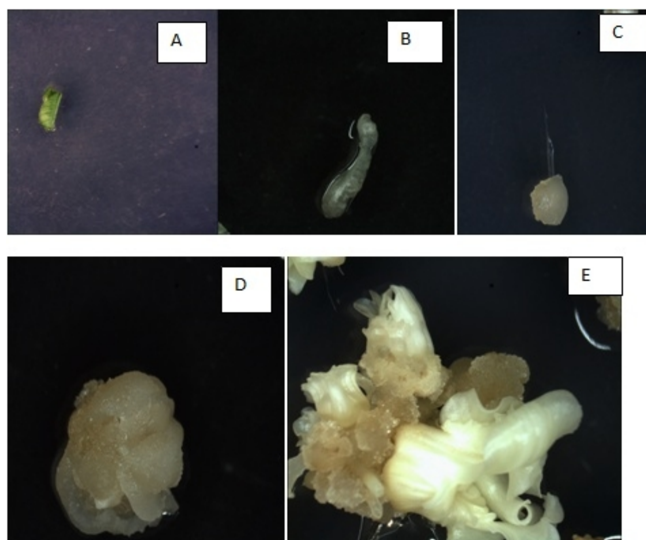


Fig. 2. Induction and maturation of primary somatic embryo using immature leaf lobe of cassava variety TME 12.
A- a decapitated leaf lobe (X5), B- 4—day old leaf lobe in induction medium (X1), C- two-week old callus (x2), D- three-weeks old embryogenic callus (x2) E- mature primary somatic embryo (x1)

Table 3. Induction and formation of primary somatic embryo using leaf lobes in some cassava varieties

variety	Responding leaf lobe (%)	% explants with pre-embryogenic structure	PSEF (%)	PSEF (No/explants)	Location of embryo on explant	Shape of embryo
TMS 1425	62.5±1.8^{d}	0.0^{c}	0.0^{c}	0.0^{c}	-	-
Albert	74.4±1.9^{b}	79.6±2.4^{b}	85.7±3.6^{b}	4.5±1.2^{b}	base	CC
TMS 98/0505	75.6±0.8^{b}	0.0^{c}	0.0^{c}	0.0^{c}	-	-
TMS 97/3200	60.8±1.5^{d}	0.0^{c}	0.0^{c}	0.0^{c}	-	-
TMS 98/0068	63.7±1.3^{c}	0.0^{c}	0.0^{c}	0.0^{c}	-	-
TMS 91/02324	65.1±3.2^{d}	0.0^{c}	0.0^{c}	0.0^{c}	-	-
TMS 92B/0068	68.3±2.5^{c}	0.0^{c}	0.0^{c}	0.0^{c}	-	-
TMS 97/4768	57.8±0.7^{d}	0.0^{c}	0.0^{c}	0.0^{c}	-	-
TMS 98/0510	54.3±0.8^{d}	0.0^{c}	0.0^{c}	0.0^{c}	-	-
Kibaha	72.4±2.4^{c}	77.4±1.5^{b}	88.5±2.7^{b}	5.6±0.5^{a}	even	FC
TME 12	87.5±3.2^{a}	84.6±2.3^{a}	93.4±4.3^{a}	4.5±0.6^{b}	top	SC
Mean	73.42	26.17	44.63	2.43	-	
CV (%)	6.77	164.3	106.92	114.81	-	

Values are means (±standard error). Values followed by different superscripts in same column are significantly different at P≤0.05 by Duncan's multiple range test. CV= coefficient of variation C=conical cotyledon FC=fused cotyledon SC-separated cotyledon

Comparing somatic embryo production of immature leaf lobe explant with axillary meristem explant in the three landraces that produced mature primary somatic embryo, primary somatic embryogenesis frequencies and efficiencies in immature leaf explant were greater than in axillary meristem explant by an average of 86.1% and 82.7%, respectively.

4. DISCUSSION

Production of somatic embryo is critical for development of transgenic cassava plants because somatic embryo is the often used target tissue for insertion of foreign genes [13]. In this study, eleven varieties were screened for induction and maturation of primary somatic embryo using immature leaf lobe and axillary meristem. Out of the 11 varieties screened for primary somatic embryogenesis using LL explant, only three varieties (Albert, Kibaha and TME 12) produced mature somatic embryo, which are landraces. Previously, only three varieties (TMS 4 (2) 1425, TME 12 and TMS 91/02324) have been screened for somatic embryogenesis using LL and APM explants (Table 1). Out of the three varieties, mature primary somatic embryos were obtained in TME 12 and TMS 91/02324 varieties from both LL and shoot apical meristem explants (Hankoua et al., 2005). TMS 4 (2) 1425 formed only pre-embryogenic structures and failed to develop to mature embryo. The PSEF and PSEE observed in the present study are comparable with previous reports. A range of 88.5 to 93.6% and 4.5 to 5.6 embryos per explant were observed in this study as PSEF and PSEE, respectively in the three varieties that produced primary somatic embryo. Hankoua et al. [20] reported a range of 48-100% in PSE frequency for a wide range of cassava varieties. Specifically, PSEF (93.6%) and PSEE (4.5 embryo per explant) for TME 12 using LL explants observed in the present study were comparable to PSEF (94.0%) and PSEE (3.7 embryos per explant) reported by Hankoua et al (2005). Recently, a range of primary somatic embryogenesis frequencies of 4 to 89% was reported using LL as explant on picloram-based medium in eight cultivars of South African origin by [24]. For the same

cultivars, the efficiency of primary somatic embryogenesis ranged between 1.5 and 4.0 using LL as explants.

This study is the first documented attempt at producing primary somatic embryo from axillary meristem in cassava in a wide range of cassava varieties. Though limited variation existed in enlargement of node among cassava varieties, formation of pre-embryogenic structure was largely under genetic influence. Similar reports were made on formation of pre-embryogenic structure from immature leaf lobe in cassava [20,21]. The successful enlargement of node in culture media does not translate to induction of pre-embryogenic structure. However, there appear to be a strong relationship between formation of pre-embryogenic structure and maturation of primary somatic embryo as 75% of varieties that formed pre-embryogenic structure produced mature primary somatic embryo. In the present work, the PSEF ranged from 72.7% to 93.6%. Using shoot apical meristem as explant, the frequency of primary somatic embryogenesis were 77 and 93% for TME 12 and TMS 91/02324, respectively as reported by [20]. Feitosa et al. [27] reported 15.5 -80.0% frequency of primary somatic embryo and 18.9-24.4 embryo per explant in some cassava genotypes of Brazil, using shoot tip explant on picloram-supplemented medium. The successful production of primary somatic embryo using axillary meristem as explant in some cassava varieties established the fact that somatic embryo can be obtained from lateral meristem, apart from apical meristem which had earlier been reported by [20,28,29,30]. Mature primary somatic embryo were produced in landraces (Albert, Kibaha and TME 12) while pre-embryogenic structure was obtained only in one improved variety (TMS 98/0505), which did not develop to mature embryo. This result suggested that picloram-based induction medium may be suitable for cassava landraces. Recalcitrance of improved cassava varieties to induction and maturation of primary somatic embryo has been reported [20]. The result suggested that variety also influenced the location of the embryo on explant and shape of the embryo.

5. CONCLUSION

The greater values of PSEF and PSEE of LL explant than AM explant suggest that the use of immature leaf lobe explant has potential for production of a large number of explants for secondary embryogenesis, regeneration and transformation studies.

ACKNOWLEDGEMENT

This paper is a portion of the Ph.D Thesis of the first author. The offer of Visiting Research Fellowship to the first author by the International Institute of Tropical Agriculture (IITA), Ibadan is appreciated. The financial support of Obafemi Awolowo University's Research Committee is acknowledged.

COMPETING INTERESTS

Authors declare that there are no competing interests between individuals and organizations that can affect the publication of this work.

REFERENCE

1. Defloor I, Dehing I, Delcour JA. Physico-chemical properties of cassava starch. Trop Sci. 1998;31:189-207.

2. Nweke FI, Spencer DDC, Lynam JK. The cassava transformation, Michigan State University Press, East Lansing; 2002.
3. El-Sharkawy MA. Cassava biology and physiology. Plant Mol. Biol. 2004;56:481–501.
4. Fermont AM, Babirye A, Obiero HM, Abele S, Giller KE. False beliefs on the socio-economic drivers of cassava cropping. Agron Sustain Dev. 2010;30:433–444.
5. Fermont AM, van Asten PJA, Tittonell P, van Wijk MT, Giller KE. Closing the cassava yield gap: an analysis from smallholder farms in East Africa. Field Crops Res. 2009;112:24-36.
6. Sanchez T, Salcedo E, Dufour D, Morante N, Debouck D, Moreno IX. Screening of starch quality traits in cassava (*Manihot esculenta* Crantz). Starch/ Starke. 2009;61:12-19.
7. Blennow A. Towards predictable functionalization of starch. Starch/Starke. 2003;50:58-64.
8. Raemakers K, Schreuder M, Suurs L, Furrer-Verhorst H, Vincken J-P, de Vetten N, Jacobsen E, Visser RGF. Improved cassavastarch by antisense inhibition of granule-bound starch synthase I. Mol Breed. 2005;16:163–172.
9. Ceballos H, Iglesias CA, Pereze JC, Dixon AGO. Cassava breeding: opportunities and challenges. Plant Mol Bio. 2004;56:503–516.
10. Barceloux DG. Cyanogenic foods (cassava, fruit kernels, and cycad seeds). Dis Mon. 2009;55:336–352.
11. Bull SE, Ndunguru J, Gruissem W, Beeching JR, Vanderschuren H. Cassava constraints to production and the transfer of biotechnology to African laboratories. Plant Cell Report. 2011 DOI 10.1007/s00299-010-0986-6.
12. Nassar N, Ortiz R. Breeding cassava to feed the poor. Sci Am. 2010;302:78–84.
13. Taylor NJ, Chavarriaga P, Raemakers K, Siritunga D, Zhang P Development and application of transgenic technologies in cassava. Plant Mol Bio. 2004;56:671–678.
14. Raemakers K, Jacobsen E, Visser R. The use of somatic embryogenesis for plant propagation in cassava. Mol Biotechnol. 2000;14(3):215-21.
15. Osorio M, Gámez E, Molina S, Infante D. Evaluation of cassava plants generated by somatic embryogenesis at different stages of development using molecular markers. Elect J Biotech 2012;15:4. Available: http://dx.doi.org/10.2225/vol15-issue4-fulltext-3.
16. Atehnkeng J, Adetimirin VO, Ng SYC. Exploring the African cassava (*Manihot esculenta* Crantz) germplasm for somatic embryogenic competence. Afr J Biotech 2006;5:1324–1329.
17. Danso KE, Ford-Lloyd BV. Induction of high-frequency somatic embryos in cassava for cryopreservation. Plant Cell Rep. 2002;21:226–232.
18. Guohua M, Qiusheng X. Induction of somatic embryogenesis and adventitious shoots from immature leaves of cassava. Plant Cell Tiss Organ Cult. 2002;70:281–288.
19. Guohua M. Effects of cytokinins and auxins on cassava shoot organogenesis and somatic embryogenesis from somatic embryo explants. Plant Cell Tiss Organ Cult. 1998;54:1–7.
20. Hankoua BB, Ng SYC, Fawole I, Pouti-Kaerlas J, Pillay M, Dixon AGO. Regeneration of a wide range of African cassava genotypes via shoot organogenesis from cotyledons of maturing somatic embryos and conformity of field-established regenerants. Plant Cell Tiss Organ Cult. 2005;82:221-231.
21. Taylor NJ, Masona MV, Carcamo R, Ho T, Schöpke C, Fauquet CM. Production of embryogenic tissue and regeneration of transgenic plants in cassava (*Manihot esculenta* Crantz). Euphy. 2001;120:25–34.
22. Li HQ, Guo JY, Huang YW, Liang CY, Liu HX, Potrykus I, Puonti-Kaerlas J. Regeneration of cassava plants via shoot organogenesis. Plant Cell Rep. 1998;17:410–414.

23. Raemakers KJJM., Sofiari E, Jacobsen E, Visser RGF. Regeneration and transformation of cassava. Euphy. 1997;96:153–161.
24. Rossin CB, Rey MEC. Effect of explant source and auxins on somatic embryogenesis of selected cassava (*Manihot esculenta* Crantz) cultivars. South Afri J Bot. 2011;77:59 –65.
25. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 1962;15:473–497.
26. Gomez KA, Gomez AA. Statistical Procedures for Agricultural Research (2nd ed). John Wiley and Sons Inc. New York. 1984;680.
27. Feitosa T, Bastos JLP, Ponte LFA, Jucá TL. Campos FAP Somatic embryogenesis in cassava genotypes from the northeast of Brazil. Brazilian Arch Biol Techn. 2007;50:201–206.
28. Zhang P, Phansiri S, Puonti-Kaerlas J Improvement of cassava shoot organogenesis by the use of silver nitrate in vitro. Plant Cell Tiss Organ Cult. 2001;67:47–54.
29. Zhang P, Pounti-Kaerlas I. Regeneration of transgenic cassava from transformed embryogenic tissues. Methods in Molecular Biology. 2004;286:1025-1029.
30. Saelim L, Phansiri S, Netrphan S, Suksangpanomrung M, Narangajavana J. Optimization of in vitro cyclic somatic embryogenesis and regeneration of the Asian cultivars of cassava (*Manihot esculenta* crantz) for genetic manipulation system. Global J Biotech Biochem. 2006;1:7–15.

© 2013 Opabode et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=217&id=11&aid=1210