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Evaluation of Ten Elite Nigerian Cassava (*Manihot esculenta* Crantz) Cultivars for Somatic Embryogenesis and Regeneration Potentials

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FAN, EEE-O and NJT designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors EEE-O and CNE managed the analyses of the study. Author FAN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Development of an efficient regeneration system is one of the basic requirements for cassava genetic transformation. Ten elite Nigerian cassava cultivars consisting of seven improved genotypes and three local landraces were evaluated for their ability to produce somatic embryos *in vitro*. Responses of these cassava cultivars were compared with that of a model cultivar TMS 60444 known for its potential in somatic embryo production. Somatic embryogenesis in cassava is variety dependent probably due to its recalcitrant nature to *in vitro* manipulations. Production of somatic embryos in cassava is of two kinds – organized embryogenic structure (OES) and friable embryogenic callus (FEC). All the ten cassava cultivars screened produced organized embryogenic structures on DKW induction medium supplemented with 20 g/l sucrose, 8 g/l Difco noble agar and

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50 µM Picloram. Friable embryogenic callus generation in cassava is usually achieved by subsequent transfer of the high quality OES formed to another induction medium Gresshoff and Doy (GD) medium supplemented with 20 g/l sucrose, 50 µM Picloram and solidified with 8 g/l Difco noble agar. Out of the ten cassava cultivars screened only four possessed the capacity for FEC production. Among the ten cassava cultivars screened for somatic embryogenesis, more than half of them had the potential for plantlet regeneration from the two types of somatic embryos.

Keywords: Friable embryogenic callus; in vitro; manihot esculenta; organized embryogenic structure; picloram; somatic embryogenesis.

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) belongs to the family Euphorbiaceae. It is one of the most important staple food crops in Africa. It derives its importance from the fact that its starchy, thickened tuberous roots are valuable source of cheap calories, especially in developing countries where calories deficiency and malnutrition are widespread [1]. Africa accounts for most of the cassava root harvested worldwide with more than half of the world's total production [2] followed by Asia and Latin America. Cassava apart from its importance as animal feed and industrial raw material has emerged as an important biofuel resource [3].

In Africa, cassava production on large scale is limited as a result of inherent problems associated with the crop. It is a highly heterozygous and clonally propagated crop and some of the varieties do not flower [4]. The crop is highly susceptible to cassava mosaic diseases (CMDs) such as African Cassava Mosaic Virus (ACMV), East African Cassava Mosaic Virus (EACMV) as well as Cassava Brown Streak Virus (CBSV).

Application of conventional methods of cassava breeding is not enough to provide the needed solutions to the problems confronting cassava production. There is therefore the need to combine modern breeding techniques with traditional breeding methods in order to achieve the desired breakthrough as implicated in other crops like maize [5], rice [6] and barley [7].

One of the requirements for the generation of genetically engineered cassava is an efficient and reproducible plant regeneration and transformation system. Most of the African cassava cultivars are recalcitrant to methods of plant genetic transformation. Cassava genetic transformation appears to depend on the capacity of each genotype to generate somatic embryos on induction medium which are the target tissues for transgene insertion *via Agrobacterium*-mediated transformation.

Transgenic cassava was first reported in 1996 [8; 9]. Since then three different genetic transformation systems have been developed, which depended on the initial induction of somatic embryogenic structures from immature leaf explants [10]. Such structures can be manipulated to generate totipotent friable embryogenic callus, or to produce mature green cotyledon structures. Somatic embryogenesis is the method of choice in regeneration of cassava and explants have been mostly restricted to young leaves and shoot meristems [11].

There are more than 1500 cassava cultivars worldwide available [12]. Studies have shown that only few cultivars have been efficiently transformed namely TMS 60444 [9,13,14,15], TMS 71173 [16], MCol 2215 [17; 18; 19], MCol 122 [13,14] and MPer 183 [20]. A high-throughput platform have been developed for production of transgenic cassava for the cultivar TMS 60444 [21]. This has led to the production of transgenic TMS 60444 expressing traits for nutritional enhancement and disease resistance [21]. Nigeria is the world's largest producer of cassava and still it is not enough to meet the demand of the nation's teeming population. Biotechnology and molecular biology approaches seem to provide the solution to address these problems.

Production of somatic embryos by cassava cultivars remain the most acceptable method cassava responds to transformation either by particle bombardment or *via Agrobacterium*-mediated transformation. Most of the Nigerian cassava cultivars have not been assessed for their abilities to generate good quality embryos displaying the characteristics necessary for transgene incorporation. There is the need to screen each of the Nigerian cassava cultivars for somatic embryo generation. This study was carried out to evaluate competence of ten

Nigerian cassava varieties to somatic embryogenesis as well as to regenerate somaclonal plantlets.

2. MATERIALS AND METHODS

2.1 Planting Material

The planting materials used for this research consisted of ten (10) cassava cultivars comprising seven (7) improved genotypes that were obtained from Cassava Program Unit and three (3) promising farmer's preferred local landraces that were collected from farmers in Abia State. The improved genotypes used for this study included: TMS 97/2205, TMS 98/0505, TMS 98/0510, TMS 98/0581, TME 419, TMS 96/1632 and NR 87184 while the three local landraces were ''Nwibibi'', ''Nwugo'' and ''Okwuoto''. .

2.2 Type of Explants

The explants used for *in vitro* cultures were derived from the cassava cuttings obtained from the field. These cassava cuttings from the ten cultivars were planted out in plastic pots and allowed to grow for two months. These potted plants served as source of mother plants from which nodal cuttings (explants) were collected for initiation *in vitro*.

2.3 Study Area

The *in vitro* plantlets used for the study were raised at the tissue culture laboratory at the National Root Crops Research Institute (NRCRI), Umudike. The *in vitro* cultures consisted of ten cassava cultivars (seven improved genotypes and three local landraces), which were later sent to the USA in 2011 for somatic embryogenesis study. The *in vitro* study on responses of the ten cassava cultivars to somatic embryogenesis was carried out at the International Laboratory for Tropical Agricultural Biotechnology (ILTAB), Donald Danforth Plant Science Center, St. Louis, Missouri, USA.

2.4 Plantlets Multiplication Via Sub-Culturing

Murashige and Skoog (MS) modified medium was employed in the multiplication of the various cassava plantlets by sub-culturing on MS basal medium supplemented with 20 g/l sucrose (MS2 Agar) using sterile disposable Petri dishes to obtain enough plantlets to supply immature leaf lobes needed for callus induction.

2.5 Culture Media Composition/ Preparation

Murashige and Skoog (MS) [22] and Gresshoff and Doy (GD) [23] basal media were prepared from constituent chemicals (Sigma Chemical Co., St. Louis) http://www.phytotechlab.com/. Macro- and micronutrients, vitamins and Fe- EDTA stock solutions are prepared at 25x, 1000x, 1000x and 200x concentrations respectively and stored at 4°C until required. Stocks were mixed, sucrose added at 20 g/l, pH adjusted to 6.1 with 1N NaOH and Difco Noble Agar added at 8 g/l before autoclaving at 12ºC for 20 min. Naphthalene acetic acid (NAA) and benzylaminopurine (BAP) were added prior to autoclaving but Picloram and antibiotics when needed were added from filter sterilized stock solutions after media was autoclaved and cooled at 42ºC, as determined by the use a FLUKE 62 Mini IR Thermometer (Fluke Biomedical, Everett, WA). All GD and MS based media containing Picloram were dispensed into 9 cm x15 mm plates at 25 ml per dish while the MS based regeneration and whole plant media were poured into 10 cm x 25 mm plates at 40 ml per Petri dish.

2.6 Induction and Maturation of Somatic Embryos

Immature leaf lobes derived from six weeks old *in vitro* grown plantlets were used for callus induction. The unexpanded leaves of these explants were cultured on 5.22 g/l Driver and Kuniyuki Walnut (DKW) medium [24] supplemented with 20 g/l sucrose, 50 µM Picloram and solidified with 8 g/l Difco noble agar. The pH was adjusted to 5.7 ± 0.1 before autoclaving at 121ºC for 15 minutes. 25 ml of the media were poured into 9 cm x15 mm plates under a sterile laminar flow hood and allowed to cool and solidify.

The immature leaf lobes were extracted with the aid of a dissecting microscope, using a sterile forceps, syringe and needle. The leaf lobes were exposed with forceps, excised with the needle and placed on media with the abaxial side and the midrib of the explants placed in contact with the medium. Ten Petri dishes were raised for each cassava cultivar with each Petri dish having

12 pieces of leaf lobe explants plated on it except cultivars with limited supply leaf lobes. The cultured Petri dishes were incubated under dim light at 28±2ºC for 21 to 30 days before transferring them to another induction medium – Gresshoff and Doy (GD) medium for generation of friable embryogenic callus.

2.7 Production of Friable Embryogenic Callus (FEC)

Friable embryogenic callus was established from high quality three week old organized embryogenic structures (OES) formed from DKW medium following the method described by [25]. These embryogenic tissues were cultured on Gresshoff and Doy medium modified by addition of 20 g/l sucrose, 50 µM Picloram (GD2 50P) and solidified with 8 g/l Difco noble agar. The Picloram (synthetic growth regulator) was added to the medium after allowing it to cool to a temperature of 45ºC under a laminar flow hood. Clusters of OES were placed on GD2 50P medium with each Petri dish receiving a total of five clusters. The cultured plates were incubated in the dark or dim light at a temperature of 28±2ºC.

Somatic embryos from each of the ten cassava genotypes plus TMS 60444 cultivar were cultured and assessed for their abilities to produce friable embryogenic callus on GD2 50P medium on three different occasions. Each plate received cluster of somatic embryos into five different places and this was replicated five times.

The meshing of the embryos using a mesh to obtain uniform sized embryos was also tried. The meshed embryos were incubated in the dark at temperature of 28ºC for five days and subsequently transferred to fresh media and to be subcultured every 2-3 weeks.

Also investigated was the effect of different concentrations of Picloram (50 µM and 100 µM) on three selected cassava genotypes (TME 419, TMS 96/1632 and 'Okwuoto'). The trial was conducted following the same method explained above but altering the Picloram concentrations.

2.8 Maturation, Germination of Somatic Embryo and Plantlet Recovery

The two kinds of somatic embryos – organized embryogenic structures (OES) and friable embryogenic callus (FEC) were transferred separately to stage 1 regeneration medium (MS2 5NAA) without antibiotics for maturation of the embryos. Stage 1 regeneration media consist of Murashige and Skoog salts and vitamins supplemented with 20 g/l sucrose, 5 µM α-naphthalene acetic acid (NAA) and solidified by 8 g/l Difco noble agar. This was followed by subsequent transfer to stage 2 regeneration medium (MS2 0.5NAA) in which the concentration of the NAA was reduced to 0.5 µM after three weeks. The plates containing the tissues were taken to the growth room and incubated at 28ºC with 16 hours light and 8 hours darkness for maturation into cotyledons.

After three weeks the cotyledons that developed from the stage 1 or stage 2 were further moved to germination medium (MS2 2BAP). This medium consisted of MS based salts and vitamins modified by 2 µM 6-benzylaminopurine (BAP) in place of NAA. Somatic embryos that have developed to possess two distinct, green cotyledons, a swollen hypocotyls and meristem region were transferred to the germination medium making sure that any adhering callus was removed and the embryos pressed into the medium such that the downside of the cotyledons were in contact with the medium without being submerged.

After three to four weeks germinated cotyledonary plantlets that have developed expanded leaves were transferred to Murashige and Skoog basal medium (MS2 agar) for rooting and further shoot development.

3. RESULTS AND DISCUSSION

The *in vitro* raised cassava cultures at the tissue culture laboratory of National Root Crops Research Institute, Umudike and the *in vitro* culture plates undergoing multiplication at the tissue culture laboratory in Danforth Plant Science Center are shown in Fig. 1.

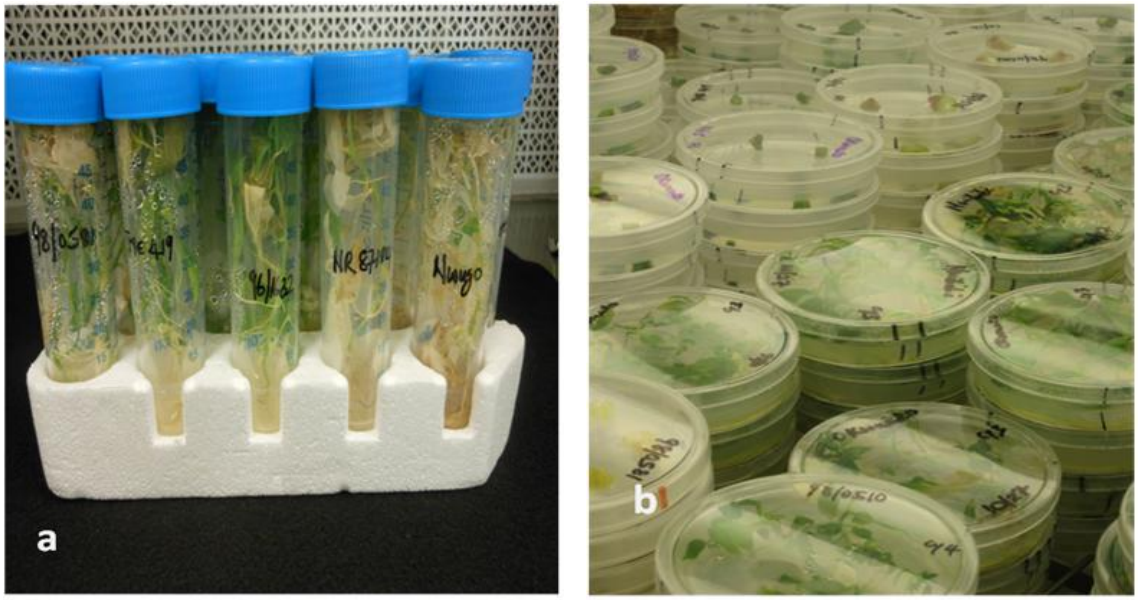

Fig.1. *In vitro* raised cassava plantlets exported to Danforth center, USA (a); *In vitro* cassava cultures undergoing multiplication at the tissue cultures lab in Danforth center

Fig. 2 shows pictures of somatic embryos (OES) produced by the Nigerian cassava cultivars evaluated for somatic embryogenesis. All the ten Nigerian cassava cultivars produced organized embryogenic structures on DKW medium used in this study for somatic embryo production (Table 1). Responses of cassava to somatic embryogenesis are variety dependent as reported by [16,26,9,19]. In this study, somatic embryo induction efficiency varied depending on the genotype with the model cultivar TMS 60444 (control) given the highest induction efficient as well as average amount of OES produced per explants (Table 1). Among the ten Nigerian cassava varieties screened, six of them scored above 50% with TMS 96/1632, 'Okwuoto' and 'Nwibibi' scoring 66, 65 and 64% respectively.

Similar observation was made by [27] in their study on responses of two Asian cassava cultivars to effects of different phytohormones.

Formation of FEC using GD2 50P medium is difficult to achieve in some varieties due to recalcitrant nature of cassava. In some cassava varieties, FEC production is induced when GD2 50P medium is supplemented with 250 or 500 µM tyrosine. Similarly, meshing of the organized embryogenic structures through a mesh to obtain a uniform size before plating on GD media has been shown to promote FEC generation in some cultivars. Formation of FEC was achieved in some cultivars through sub-culturing of the cultured somatic embryos every 2 to 3 weeks with minimal production of non embryogenic callus [28;29]. Some cassava cultivars could not produce FEC irrespective of the number of trials even with the addition of tyrosine to the GD2 50P medium. In this study, FEC formation was made possible in some cultivars when the somatic embryos were meshed and cultured on GD2 50P modified by addition of 250 µM or 500 µM tyrosine.

Contrasting to what were observed in the production of OES among the ten cultivars, production of FEC were achieved in only four cultivars consisting of two improved lines TME 419 and TMS 96/1632 (Fig. 3) and two local lines 'Okwuoto' and 'Nwugo' (Fig. 4). In cassava genetic transformation friable embryogenic callus is the preferred target tissues for transgene insertion either by *Agrobacterium*-mediated transformation [30,31,32,24,21] or by particle bombardment [31,9,32,15,14].

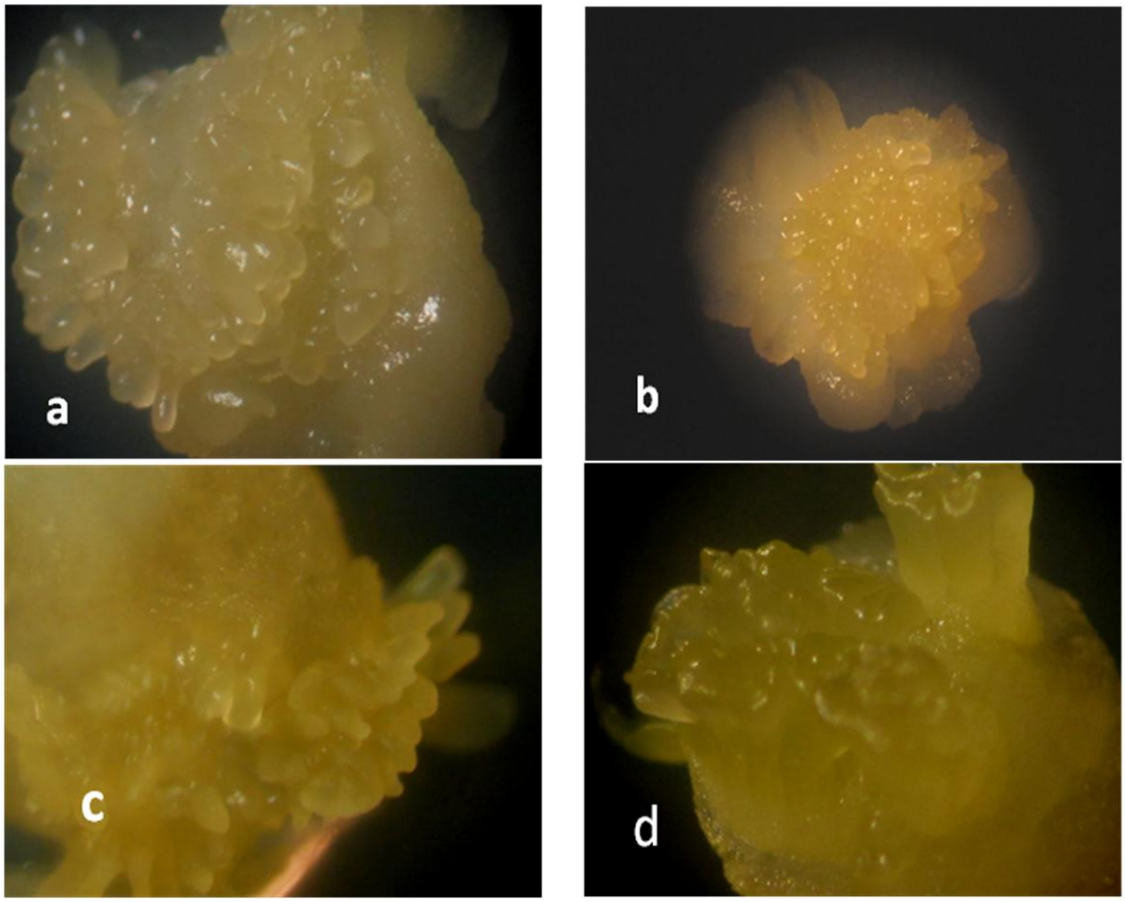

Fig. 2. Pictures of OES produced by (a) TMS 96/1632; (b) TMS 419; (c) TMS 98/0581 and (d) Okwuoto varieties on DKW supplemented with 50µM Picloram and incubated under low light intensity. (Mag. X40)

Table 1. Summary of somatic embryo (OES) production by the ten Nigerian cassava cultivars evaluated using a model cultivar TMS 60444 as basis for comparison

Variety	Somatic embryo (OES) / Total explants	Embryo induction frequency (%)	Average amount of OES / Explant
TMS 98/0510	30/72	42	0.55
TMS 98/0505	44/84	52	0.61
TMS 98/0581	47/84	56	0.71
TMS 97/2205	26/66	40	0.52
TMS 96/1632	55/84	66	0.9
TME 419	21/63	33	0.39
NR 87184	41/72	57	0.69
'NWIBIBI'	54/84	64	1.01
'NWUGO'	03/36	10	0.11
'OKWUOTO'	54/84	65	0.89
TMS 60444	67/84	80	1.25

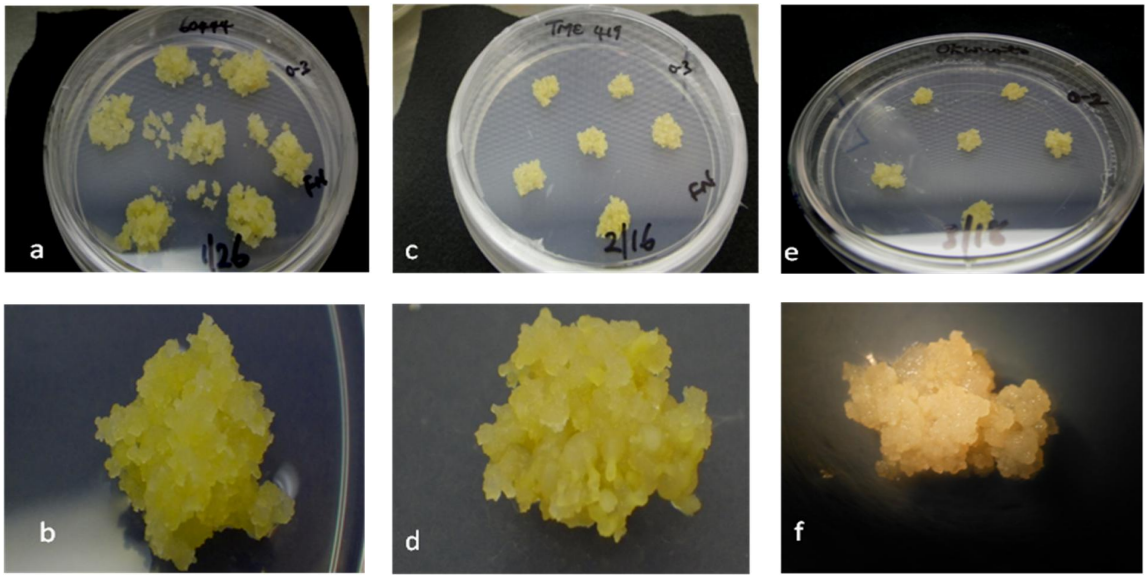

Fig. 3. Pictures of FEC generated by TMS 60444 (a & b) on GD 2 50P, TME 419 (c & d) on GD 2 50P modified with 250 µM thyrosine and Okwuoto (e & f) on GD 2 50P modified with 250 µM thyrosine. (Mag. X40)

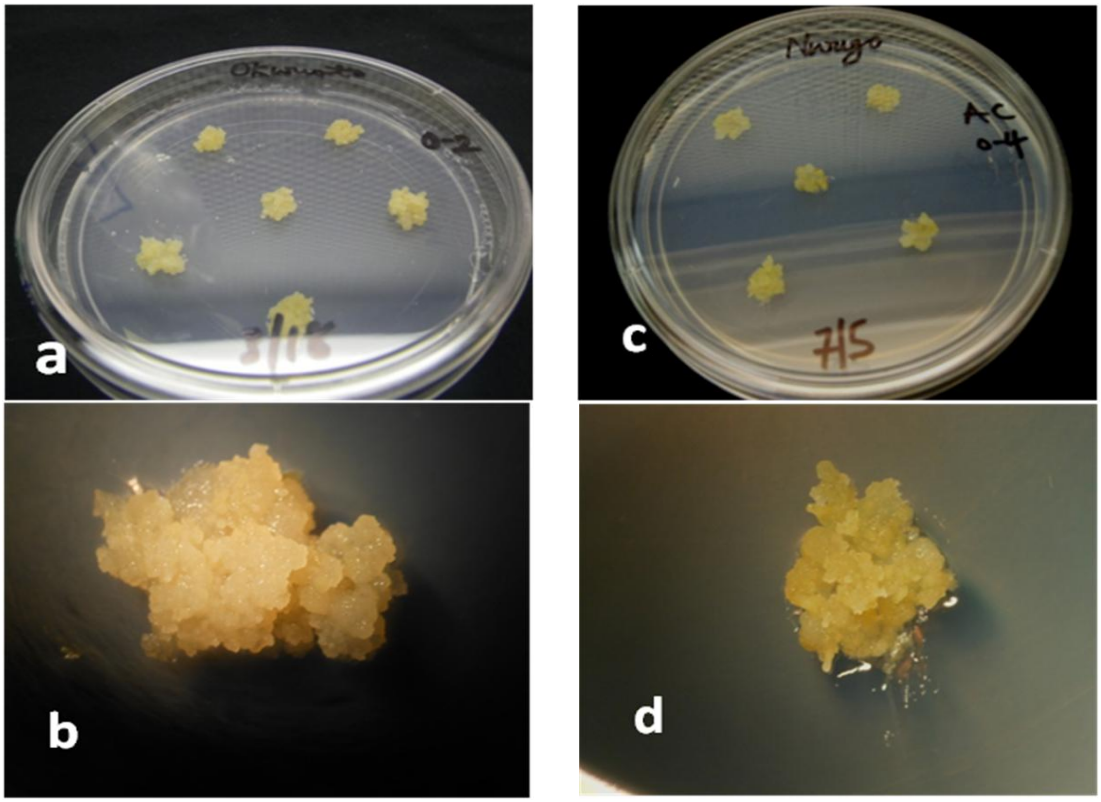

Fig. 4. Pictures of FEC produced by two Nigerian local lines 'Okwuoto' (a and b) on GD medium modified with 50 µM Picloram and 250 µM tyrosine and 'Nwugo' (c and d) on GD medium modified with 50 µM Picloram alone. (Mag. X40)

We tried the regeneration capabilities of these somatic embryos produced by the ten Nigerian cultivars into plantlets through their subsequent transfer to different stages of regeneration media as described by [21]. Studies have shown that both OES and FEC are capable of regenerating to plantlets when transferred into appropriate regeneration medium [33]. In our study we succeeded in regenerating cassava plantlets from six cultivars out of the ten Nigerian cassava varieties evaluated (Fig. 5). Table 2 summarizes the performances of the ten Nigerian cassava varieties in terms of OES, FEC generation and plantlet regeneration.

For reliable and efficient transformation system to be achieved in any cassava cultivar there was the need to ascertain the regularity and consistency in the ability of each cassava cultivar to generate somatic embryo especially FEC when cultured in an induction medium. Based on this, six selected cassava cultivars among the ten Nigerian cultivars were further screened in order to verify their potential for FEC production. These six cultivars were selected based on their abilities to produce immature leaf lobes (explants) and good quality organized embryogenic structures. All the six cassava cultivars produced high frequencies of OES but FEC production was achieved in only two varieties (Table 3).

TME 419 and 'Nwugo' varieties which produced FEC in initial experiment (Table 2) were excluded from this screening because they could not meet the criteria used for the selection. This observation also confirms the observations made by other researchers that somatic embryogenesis in cassava are variety dependent as reported by [34,30] and [28,35]. This suggests that more studies are needed to explore somatic embryogenesis for each cassava cultivar as well as optimization of media for FEC formation.

In conclusion, this study has confirmed the recalcitrant nature of cassava to somatic embryogenesis as reported by other researchers. On the other hand, the study has shown that every cassava cultivar (improved or local) possesses the potential to generate OES as well as FEC if subjected to appropriate *in vitro* conditions it requires.

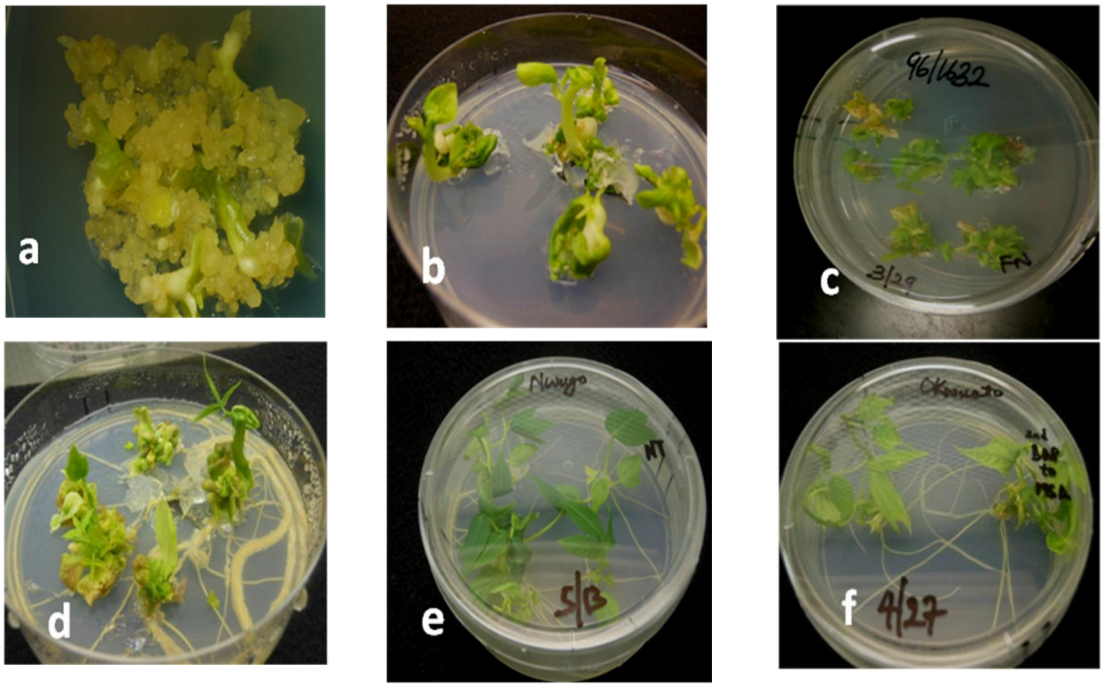

Fig. 5. Pictures showing (a) germinating embryos via FEC on MS2 0.5 NAA, (b-d) developing cotyledons on MS2 2BAP and (e-f) regenerated plantlets rooting on MS2 agar medium. (Mag. X40)

Table 2. Summary of ten Nigerian cassava cultivars assessed for somatic embryogenesis and regeneration potentials

Variety	Media used	Somatic embryo produced	[a]Plantlet regenerated
TMS 98/0510	DKW / GD2 50P	OES only	-
TMS 98/0505	DKW / GD2 50P	OES only	-
TMS 98/0581	DKW / GD2 50P	OES only	-
TMS 97/2205	DKW / GD2 50P	OES only	-
TMS 96/1632	DKW / GD2 50P + Tyrosine	OES and FEC	+
TME 419	DKW / GD2 50P	OES and FEC	+
NR 87184	DKW / GD2 50P	OES only	+
'NWIBIBI'	DKW / GD2 50P	OES only	+
'NWUGO'	DKW / GD2 50P	OES and FEC	+
'OKWUOTO'	DKW / GD2 50P + Tyrosine	OES and FEC	+
TMS 60444	DKW / GD2 50P	OES and FEC	+

[a] *+ = plantlet regenerated; - = no plantlet regenerated*

Table 3. Further screening of the six selected cassava cultivars out of the ten for OES/FEC generation

Variety	Somatic embryo (OES)/Total xplant	Embryo induction frequency (%)	Average amount of OES/Explant	Friable embryogenic callus formed
TMS 98/0505	90/120	75	0.87	No
TMS 98/0581	67/120	56	0.65	No
TMS 96/1632	84/120	70	0.96	Yes
NR 87184	94/120	78	0.87	No
'NWIBIBI'	59/120	49	0.63	No
'OKWUOTO'	74/120	61	0.84	Yes

4. CONCLUSION

[In this study, production of friable embryogenic callus (FEC) was achieved for the first time from a local Nigerian cassava cultivar "Okwuoto". This projects the cultivar as a potential candidate for genetic improvement via *Agrobacterium tumefaciens.*

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. IITA. Cassava in Tropical Africa: A reference manual. Wisbech, U.K: Bolding Mansel international. 1990;176.
2. FAOSTAT. FAO statistical databases. FAO, Rome, Italy; 2010. Available:www.faostat.fao.org
3. Egesi CN, Ogbe FO, Akoroda M, Ilona P and Dixon AGO. Resistance profile of improved cassava germplasm to cassava mosaic disease in Nigeria. Euphytica. 2007;155:215-224. DOI: 10.1007/s10681-006-9323-0.
4. Jennings DL, Iglesias C. Breeding for crop improvement. In: Cassava Biology, Production, Utilization. Hillocks RJ, Thresh JM and Bellotti AC editors. CABI Publishing, Oxon, New York; 2002.

5. Ishida Y, Hiei Y, Komari T. Agrobacterium-mediated transformation of maize. Nat. Protoc. 2007;2:1614-1621.
6. Hiei Y, Komari T. *Agrobacterium*-mediated transformation of rice using immature embryo or calli induced from mature seed. Nat. Protoc. 2008;*3*:824-834.
7. Bartlett JG, Alves SC, Smodley M, et al. High- throughput *Agrobacterium*-mediated barley transformation. Plant meth. 2008;4:22. DOI: 10, 1186/1746-4811-1184-1122.
8. Li H-Q, Sautter C, Potrykus I and Pounti-Kaerlas J. Genetic transformation of cassava (*Manihot esculenta* Crantz). Nature Biotech. 1996;14:736-740.
9. Schöpke C, Taylor N, Carcamo R, Konan NK, Marmey P, Henshaw GG, Beachy RN and Fauquet C. Regeneration of transgenic cassava plants (*Manihot esculenta* Crantz) from microbombarded embryogenic suspension cultures. Nature Biotech. 1996;14:731-735.
10. Taylor NJ, Chavarriaga P, Raemakers K *et al*. Development and application of transgenic technologies in cassava. Plant Mol. Biol. 2004;56:671-688.
11. Schöpke C, Franche C, Bougusz D, Chavarriaga P, Fauquet C and Beachy RN. Transformation in cassava (*Manihot esculenta* Crantz). In: Bajaj YPS editor. Biotechnology in agriculture and forestry, vol. 23: plant protoplasts and genetic engineering IV. Springer, Berlin Heidelberg New York. 1993;273-289.
12. Alves AAC. Cassava Botany and Physiology In: Cassava Biology, Production and Utilization. Hillocks RJ, Thresh JM, and Bellotti AC editors. CABI Publishing Oxon, New York; 2002.
13. Zhang P, Potrykus and Puonti-Kaerlas J. Efficient production of transgenic cassava using negative and positive selection. Transgenic Research. 2000a;9:405–415.
14. Zhang P, Legris G, Coulin P and Puonti-Kaerlas J. Production of stably transformed cassava plants via particle bombardment. Plant Cell Rep. 2000b;19:939–945.
15. Gonzalez AE, Schöpke C, Taylor NJ, Beachy RN and Fauquet CM. Regeneration of transgenic cassava plants (*Manihot esculenta* Crantz) through *Agrobacterium*-mediated transformation of embryogenic suspension cultures. Plant Cell Reports. 1998;17:827-831.
16. Ihemere U, Arias-Garzon D, Lawrence S and Sayre Genetic modification of cassava for enhanced starch production. Plant Biotechnology Journal 2006;4:453-465.
17. Arias-Garzon DI. Genetic engineering approaches to improve agronomic traits in cassava (Manihot esculenta Crantz). Ph.D thesis, the Ohio State University, Columbus, OH; 1997.
18. White W, Arias-Garzon D, McMahon JM and Sayre R. Cyanogenesis in cassava: The role of hydroxynitrile lyase in root cyanide production. Plant Physiology. 1998;116:1219-1225.
19. Siritunga D, Sayre RT. Generation of cyanogens-free transgenic cassava. Planta. 2003;217:367-373.
20. Sarria R, Torres E, Angel F and Chavarriaga P. Transgenic plants of cassava (*Manihot esculenta*) with resistance to Basta obtained by *Agrobacterium*-mediated transformation. Plant Cell Reports. 2000;19:339–344.
21. Taylor NJ, Gaitan-Solis E, Moll T, Trauterman B, Jones T, Pranjal A, Trembly C, Abernathy V, Corbin D and Fauquet CM. A high-throughput platform for the production and analysis of transgenic cassava (*Manihot esculenta*) plants. Tropical Plant Biol. 2012;5:127-139.
22. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia plantarum. 1962;15:473-497.
23. Greshoff PM, Doy CH. Development and differentiation of haploid Lycopersicon esculentum (tomato). Planta. 1972;107:161-170.
24. Driver JA, Kuniyuki H. In vitro propagation of paradox walnut root stock. HortScience. 1984;19:507-509.
25. Taylor NJ, Edwards M, Kiernan RJ, Davey C, Blakesley D and Henshaw GG. Development of friable embryogenic callus and suspension culture systems in cassava (*Manihot esculenta* Crantz). Nat. Biotechnol. 1996;14:726-730.
26. Taylor NJ, Clarke M and Henshaw GG. The induction of somatic embryogenesis in fifteen African cassava cultivars. In: Roca WM and Thro AM editors. Proceedings First Scientific Meeting of the Cassava Biotechnology Network, Cartagena, Colombia, 25-28 August. 1992;134-137.
27. Saelim L, Phansiri S, Netrphan S, Suksangpanomrung M and Narangajavana J (2006) Optimization of *in vitro* cyclic

somatic embryogenesis and regeneration of the Asian cultivars of cassava (*Manihot esculenta* Crantz) for genetic manipulation system. Jour. of Biotechnology and Biochemistry. 2006;1(1):07-15.
28. Bull SE, Owiti JA, Niklaus M, Beeching JR, Gruissem W and Vanderschuren H. *Agrobacterium*-mediated transformation of friable embryogenic calli and regeneration of transgenic cassava. Nature protocols. 2009;4:1845–1854.
29. Taylor NJ, Masona MV, Carcamo R, Ho T, Schopke C and Fauquet CM. Production of embryogenic tissues and regeneration of transgenic plants in cassava (*Manihot esculenta* Crantz). Euphytica. 2001;120:25-34.
30. Raemmakers CJJM, Bessmbinder JJE, Staritsky G, Jacobsen E and Visser RGF. Induction, germination and shoot development of somatic embryos in cassava. Plant Cell Tissue and Organ Culture. 1993a;33:151-156.
31. Raemekkers CJJM, Sofiari E, Taylor N, Henshaw G, Jacobsen E and Visser RGF. Production of transgenic cassava (*Manihot esculenta* Crantz) plants by particle bombardment using luciferase activity as selection marker. Molecular Breeding. 1996;2:339-349.
32. Schöpke C, Franche C, Bougusz D, Chavarriaga P, Fauquet C and Beachy RN. Transformation in cassava (*Manihot esculenta* Crantz). In: Bajaj YPS editor. Biotechnology in agriculture and forestry, vol. 23: Plant protoplasts and genetic engineering IV. Springer, Berlin Heidelberg New York. 1997;273-289.
33. Nkaa FA, Ene-Obong EE, Taylor NJ, Fauquet CM and Mbanaso ENA. Elimination of African Cassava Mosaic Virus (ACMV) and East African Cassava Mosaic Virus (EACMV) from Cassava (*Manihot esculenta* Crantz) cv. 'Nwugo' via somatic embryogenesis. American Journal of Biotechnology and Molecular Sciences. 2013;3(2):33-40.
34. Ihemere U. Somatic embryogenesis and transformation of cassava for enhanced starch production. Ph.D thesis. Ohio State University, USA; 2003.
35. Raemakers CJJM, Schavemarker CM, Jacobsen E and Visser RGF. Improvement of cyclic somatic embryogenesis of cassava (*Manihot esculenta* Crantz). Plant Cell Reports 1993b;12:226-229.

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