Special Conference Edition, November, 2018 http://dx.doi.org/10.4314/bajopas.v11i1.79S

Bayero Journal of Pure and Applied Sciences, 11(1): 497 - 501
ISSN 2006 – 6996

EFFECT OF TYPES AND CONCENTRATIONS OF AUXINS ON CALLUS INDUCTION AND PRIMARY SOMATIC EMBRYOGENESIS IN LOW CYANIDE CASSAVA CULTIVARS (*Manihot esculentum* Cranz)

Isah,[1] B.I., Mustapha,[2] Y. and Sani,[2]* L. A.
1. Department of Biological Sciences, Nigeria Police Academy Wudil, Kano, Nigeria
2. Department of Plant Biology, Bayero University, PMB 3011, Kano Nigeria, Nigeria
*Corresponding author: labdul.bot@buk.edu.ng; +234 8065 933 776

ABSTRACT
Cassava constitutes a greater part of the diet of most Africans south of the Sahara and the demand for the crops has been on a steady increase. Agriculturally, cassava performs very well, but the roots and leaves contain cyanogenic glycosides that are dangerous to human health. As such, cassava cultivars with low cyanide content offer great opportunities for domestic and industrial utilization. However the major constraints to the production of low cyanide cassava cultivar are susceptibility to diseases such as cassava mosaic virus (CMV) and non-availability of disease free planting materials. Application of biotechnology such as plant tissue culture could have a significant impact in the production large amount disease free planting materials. The effects of 2, 4-D and Picloram on callus induction and primary somatic embryogenesis in the low cyanide cassava varieties was evaluated. The result indicated a significant increase in percentage callus induction with increase in the concentration of 2, 4-D with 4mg/L producing the highest callus induction frequency of 72.21%. Similarly on media supplemented with Picloram, 7mg/L produced the highest callus induction frequency (73.11%) and frequency decreases with corresponding decrease in Picloram concentration. For the formation of somatic embryos, the result indicated a significant increase in number of primary embryos with increase in the concentrations of 2, 4-D or Picloram. This protocol presents a reproducible system for generation totipotent culture that could be used in genetic improvement of cassava and mass production of disease-free planting materials.
Keywords: Cassava, Auxin, Callogenesis, Somatic embryogenesis

INTRODUCTION

Cassava [*Manihot esculenta* (*Cranz*)] is a woody shrub belonging to the family *Euphorbiaceous*. Cassava is native to South America and is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy tuberous root. It is a major source of carbohydrates. In Nigeria, Cassava when dried to a starchy powder (or pearly) extract, is called tapioca; while its fermented, flaky version is named garri .Cassava is the third largest source of carbohydrate in the tropics (Gressholf and Doy, 1974). It is one of the most drought tolerant crops, capable of growing on marginal soils. Nigeria is the world's largest producer of cassava (Roca and Thro, 1993). Cassava is classified as sweet or bitter. Like other roots and tubers, cassava contains anti nutrition factors and toxins. Therefore it must be properly processed before consumption. Improper processing of cassava can leave enough residual cyanide to cause acute cyanide intoxication and goiters and may cause ataxia or partial paralysis (Leotard and Mckey, 2004). Among the cassava germplasm in Nigeria, there are local varieties with low cyanide content and farmers general preferred these varieties because they can be consume with minimal processing or even raw. Generation of adequate planting material of the low cyanide cassava varieties is constrained by the slow nature of conventional propagation method. Moreover, convention propagation method allows the transfer of diseases from one generation to another. Application of biotechnology in the propagation of cassava has provided a new strategy for mass production of planting materials. A number of protocols has been reported for regeneration of cassava (Ibrahim, *et al.,* 2006; Hankoua *et al.,* 2006; Wongtiem *et al.*, 2011; Ubalua and Mbanaso, 2014). Successful micropropagation of low cyanide cassava requires optimization of protocols for the existing varieties. However, no such work had been reported on the local cassava varieties cultivated in Nigeria. It is therefore, necessary to optimize the tissue culture protocol for the Nigerian varieties. The protocol could be useful for future genetic improvement and rapid propagation and dissemination of quality planting materials to farmers.

MATERIAL AND METHODS

Plant Material

Local Cassava varieties (BAKIN ICE, MESILIYA and DAKATA) used in this study were obtained from local farmers in Kano. Kano is located on the latitude 11.6°N and longitude 8.3°E with tropical type of climate characterized by two distinct seasons, wet and dry. The study was carried out in the Biotechnology laboratory, Department of Plant Science, Ahmadu Bello University Zaria, Kaduna State.

Culture medium

The medium used was Murashige and Skoog (MS) (Murashige and Skoog, 1962) basal medium consisting of macro and micro salts and vitamins. The medium was supplemented with 2% sucrose; PH was adjusted to 5.8 with 1M KOH and solidified with 8% agar before being Autoclaved for 15 minutes at 121°C. All cultures were incubated at 29±2°C.

Callus induction and development of pro-embryonic structures

Young leaves from *in vitro* plantlets were excised and cultured on MS supplemented with either 2, 3 or 4mg/L 2, 4-D or 5, 6 or 7mg/L picloram. Leaves were dissected along the midrib and placed with their adaxial part in contact with the media. Eight leaf segments were cultivated in each petri dish and six petri dishes were used for each replication. Cultures were incubated in the dark at 29±2. After four weeks of culture in the dark, frequency of callus induction and primary somatic embryos were evaluated. Data was subjected to analysis of variance (ANOVA) using SAS program (SAS, 1999) and means were separated using least significant difference Test (LSD). For percentages, the data was transformed using arcsine transformation before the analysis and were converted back to percentages for presentation.

RESULTS

Effect of Auxins on Callus Formation

When young leaves were cultured on MS supplemented with 2, 4-D or Picloram, explants were observed to swell and callus began to develop at the margins and cut edges of the young leaves. The callus formation gradually increased and covered the entire explants four weeks after sub-culture. The results indicated a significant increase in percentage callus induction with increase in the concentration of 2, 4-D. When Cassava genotypes were cultivated on media fortified with 2mg/L 2, 4-D, BAKIN ICE and MESILIYA recorded the highest response with 48.33% and 41.50% callus formation respectively. These genotypes expressed significantly higher callus induction frequencies compared to DAKATA with percentage callus induction of 30.00%. However, when 2, 4-D was increased to 3mg/L, percentage callus induction significantly increased to 58-71% (figure 1). There were also significant differences between the Cassava genotypes with respect to frequency of callus induction in the presence of 4mg/L. The highest percentage callus was produced by DAKATA and BAKIN ICE with callus induction frequency of 71.67% and 69.17% respectively. Callus induction frequency was significantly higher in these genotypes compared MESILIYA with percentage callus induction of 55.00%. On the other hand, when MS was supplemented with 5mg/L picloram, callus induction frequency was within the range of 33-48% (figure 1). Percentage callus induction increased when Picloram concentration was increased to 5mg/L (figure 1). BAKIN ICE and DAKATA recorded 48.33% and 40.00% callus induction frequencies respectively and were significantly different from MESILIYA (33.33%). Increase in the concentration of Picloram to 6mg/L did not significantly increase the frequency of callus induction (figure 1). Further increase in the concentration of Picloram to 7mg/L resulted in significant increase in the callus induction frequency (figure 1). The highest percentage callus induction was produced by BAKIN ice (83.00%) and DAKATA (80.00%) and these genotypes were significantly higher than MESILIYA (53.00%).

For primary somatic embryogenesis, the results indicated a significant increase in number of primary somatic embryos with increase in the concentration of 2, 4-D from 2mg/L to 4mg/L. When 2, 4-D was increased from 2mg/L to 3mg/L the numbers of primary somatic embryos significantly increased. DAKATA produced the highest number of primary somatic embryos with mean number of 28 somatic embryos and was significantly higher than BAKIN ICE and DAKATA with 23 and 24 somatic embryos respectively. Further, increase in 2, 4-D to 4mg/L, significantly increase the number of somatic embryos in all the genotypes evaluated in this study.

When media was supplemented with 5mg/L Picloram the number of somatic embryos was comparable to that 2mg/L 2, 4-D (figure 2) and increased in the concentration of Picloram to 6mg/L significantly increased the number of somatic embryos in all the cassava genotypes (figure 2). Further increase in Picloram to 7mg/L significantly increased the number of somatic embryos in MESILIYA (27) and DAKATA (20), but number of somatic embryos significantly decreased to 8 in BAKIN ICE.

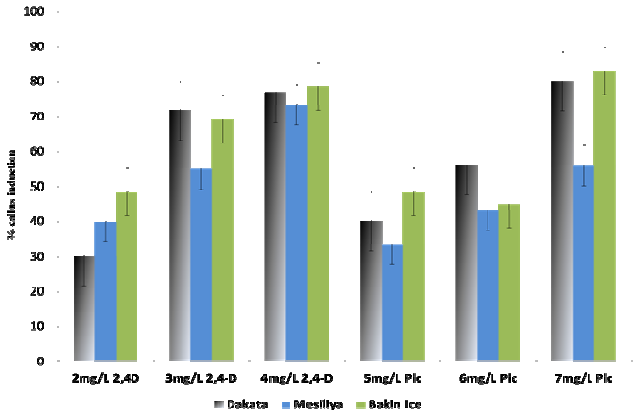

FIGURE 1: Influence of different concentration of 2,4-D and picloram on callus induction from young leaves of *in vitro* plantlets of local cassava varieties.

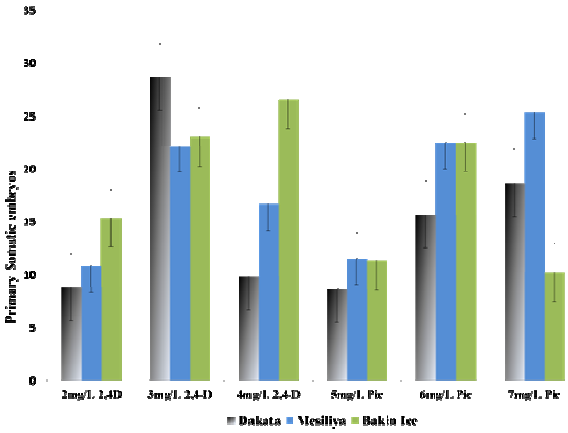

FIGURE 2: Influence of different concentrations of 2, 4-D and Picloram on the formation of primary somatic embryos in leaf derived callus of low cyanide cassava varieties.

DISCUSSION

Young leaves isolated from *in vitro* grown plants of the low cyanide cassava genotypes; BAKIN ICE, MESILIYA and DAKATA were used to determine the effect of different concentration of 2, 4-D or Picloram on callus induction and somatic embryogenesis. By 28 days after inoculation, significant number of the explants formed callus in all the cassava varieties. There were significant differences among the genotypes, type and auxin concentrations in the frequency of callus induction after four weeks of culture. The most appropriate concentrations for callus induction and somatic embryogenesis in these varieties were 4mg/L 2,4-D and 7mg/L picloram.. A number of studies have shown that picloram and 2,4-D are the growth regulators used most frequently to induce callus formation and embryogenesis in cassava (Hankoua *et al.*, 2006; Wongtiem *et al.*, 2011; Ubalua and Mbanaso, 2014).

Efficient induction of primary somatic embryos and their subsequent germination into plantlets is a pre-requisite to the production of large numbers of plantlets. Formation of primary somatic embryos was observed in all cassava varieties evaluated in this study. Production of organized pro-embryonic tissues and their subsequent development to mature somatic embryos is an essential step in somatic embryogenesis and has been reported to be genotype dependent in cassava (Raemakers *et al.*, 1997; Taylor *et al.*, 2001; Saelim *et al.*, 2006). Varietal differences in response to various concentrations of 2, 4-D and Picloram were observed in this study. Differential response of the cassava genotypes to different auxin types and concentrations were reported (Roca and Thro, 1993; Taylor *et al.*, 1996). Our study indicated that, the frequencies of primary somatic embryos in low cyanide cassava varieties can be increase with application of 4mg/L 2, 4-D. Previous reports showed that 2, 4-D was used to efficiently induced embryogenesis in *Anthirium andraeanum* (Pinheiro *et al* 2013) and sweet potato (Magalhaes *et al.*, 2006).

In addition to 2, 4-D, 7mg/L Picloram also efficiently induced somatic embryogenesis in low cyanide cassava. This finding was consistent with reports of Sofari *et al* (1997), who observed that 2, 4-D and Picloram induced somatic embryogenesis in cassava cultivars from Africa, south America and Asia. Li *et al*. (1996) and Taylor *et al*. (2001) reported that Picloram and 2, 4-D were the most efficient auxins in the induction of somatic embryos in cassava. Our finding also showed that, 7mg/L Picloram was most efficient for induction of somatic embryogenesis in low cyanide cassava. This observation is in line with findings of Raemakers *et al*. (1997) who reported that Picloram is the most suitable auxin for induction of somatic embryogenesis in African cassava. We also showed that the number of primary somatic embryos varied with the cassava varieties suggesting a strong genotype x Auxin interaction in primary somatic embryogenesis in cassava. Our findings are consistent with reports of Feitosa *et al*. (2000) and Saelim *et al*. (2006).

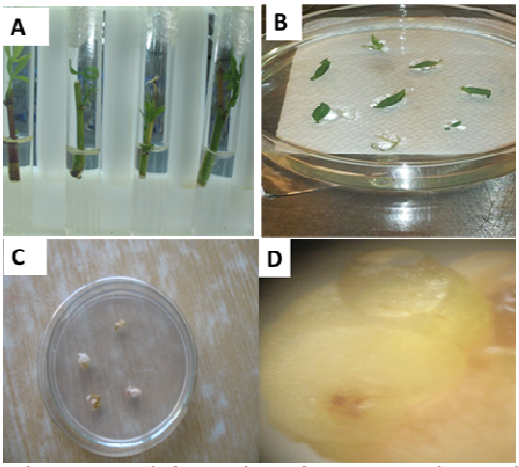

Plate 1: Stages in the development of Secondary Somatic embryos from leaf explants of low cyanide Cassava cultivars. (**A**) In vitro plantlets (**B**) Leaf sections cultured on callus induction media consisting of MS supplemented with 2, 4-D or Picloram. (**C**) Callus formation following incubation of leaf sections on callus induction media for 4 weeks in the dark. (**D**) formation of globular stage primary somatic embryos.

CONCLUSION AND RECOMMENDATION

Finally, it is worth noting that, in this study, the number of primary somatic embryos observed I this study might be related to the addition of auxin to the culture medium and the amenability of the cassava varieties to in vitro regeneration. Therefore, studies that optimized protocol for maximum production of morphological normal somatic embryos are needed. Selection of type and concentration of auxin, explant type as well as induction period are critical for production superior quality somatic embryos.

REFERENCES

Ibrahim, A.B., Heredia, F.F., Pinheiro, C.B., Aragao, F.J.L. and Campos, F.A.P., (2006). Optimization of somatic embryogenesis and selection regimes for particle bombardment of friable embryogenic callus and somatic cotyledons of cassava (*Manihot esculenta* Crantz). *African Journal of Biotechnology* Vol. 7 (16), pp. 2790-2797

Feitosa, T., Bastos, J.L. P., Ponte, L. F.A., Juca, T. L. and Campus, F. A. P., (2007). Somatic embryogenesis in Cassava genotypes from the north east of Brazil. *Braz. Arch. Biol. Technol*. 50:201-206.

Gressholf, P. M. and Doy, C. H., (1974). Description of a haploid cell line from *vitis visiflora* and importance of meiotic development of anthers for haploid culture of this and other genera. *planzenphysiologie 50:132-141.*

Léotard, G. and McKey, D., (2004). Phylogeography and origin of domestication of *cassava :insights from G3pdh sequence data from cassava and wild relatives in the Guianas. Poster presented at the 6th International Scientific Meeting of the Cassava Biotechnology Network,8-14 March 2004, CIAT, Cali, Colombia.*

Li, H-Q., Sauther, C., Potrykus, L., Puonti, K. J., (1996). Genetic transformation of Cassava (*Manihot esculenta* Cranz) *Nat. Biotechnol*. 736-740.

Magalhaes, J. S., Sentors, M. D. M., Cunha, F. N., Blumer, L., Guerra, M. P., Torres, A. C., (2006) Inducaode Embriogenese Somatica Em genotipos de batata-doce. *Horic Bras*. 24: 79-83.

Murashige, T. and Skoog, F., (1962). A revised medium for rapid growth of bioassays with *tobacco tissue cultures phisiol pl. 15:473-497*.

Pinheiro, M. V. M., Martins, F. B., de Caralho, A., Ventrella, M. C. and Otoni, W. C., (2013). Maturation of *Anthurium andranum* cv. Eidibel somatic embryos from nodal segment. *In vitro cell dev. Biol Plant*. 49:304- 312.

Raemakers, K., Jacobsen, E. and Visser, R., (2000). The use of somatic embryogenesis for plant propagation in Cassava. *Mol. Biotechnol*. 14: 215-221.

Roca, W. M. and Thro, A. M., (eds) (1993) Proceding of the first internatsional Scientific Metting of the Cassava Biotechnology Network. *Working document* 123. CIAT.

Saelim, L., Phensiri, S., Netphan, S., Suksan P. M., Narangejavana J., (2006). Optimistion of *in vitro* cyclic somatic embryogenesis and regeneration of the Asian cultivars of Cassava (*Manihot esculentum* Cranz) for genetic manipulation system. *Glob J. Bioltechnol*. 1:7-15.

SAS. (1999). Institute Inc. *SAS* Language reference. Version 6, Cary NC: *SAS Institute Inc*. 1042.

Sofari, E., Raemakers, C. J. M., Kanju, E., Danso, K., Vanlameran, A. M., Jacobsen, E. and Visser, R. G. F., (1997). Comparison of NAA and 2, 4-D induced somatic embryogenesis in Cassava. *Plant cell tissue organ culture*. 1997; 50:45-56

Taylor, N. J., Edwards, M., Kiernan, R., Devey, C. D. M., Blakesley, D. and Henshaw, G. G., (1996) Development of friable embryogenic callus and embryogenic suspension culture system in cassava (*Manihot esculenta Crantz). Nature Biotecnology* 14:727-730.

Ubalua, A. O. and Mbanaso, E., (2014). Somatic embryogenesis in two Nigerian Cassava Cultivars (Sandpaper and TMS 6444). *Journ al of Evolutin ary B iology Research*, Vol 6 (3) pp 9-12.

Wongtiem, P., Courtois, D., Florin, B., Juchaux, M., Peltier, D., Broun, P. and Ducos, J.P., (2011). Effects of cytokinins on secondary somatic embryogenesis of selected clone Rayong 9 of *Manihot esculenta* Crantz for ethanol production. *African Journal of Biotechnology* Vol. 10(9), pp. 1600-1608