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IN-VITRO PROPAGATION OF MANIHOT ESCULENTA CRANTZ THROUGH NODAL CULTURES

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ABSTRACT

Murashige and Skoog (MS) medium was supplemented with Naphthalene Acetic Acid (NAA) *and 6-benzylaminopurine BAP) in varied concentrations to determine the optimum concentration of the plant hormones needed for in vitro nodal proliferations and elongation of cassava plantlets.* Regenerated cassava nodal plantlets were obtained from International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State. The nodal plantlets were excised and sub-cultured into different test-tubes containing MS medium supplemented with NAA and BAP at different concentrations of 0.005ml NAA + 0.02ml BAP, 0.01ml NAA + 0.05ml BAP, 0.015ml NAA + 0.08ml BAP and a control without any hormone for nodal proliferations and plantlet height. The results obtained showed that the control experiment not supplemented with hormones had the highest average number of nodes (40.33) at 9 weeks, while the MS supplemented with hormones at 0.01ml NAA + 0.05ml BAP yielded the longest plantlet (23.30mm) at 9 weeks. This study revealed that hormones in varied concentrations are necessary for early germination and elongation of cassava plantlets height but nodal proliferation of the cassava plantlets is not dependent on hormone supplements.

Keywords: Cassava, Murashige and Skoog, propagation, *in vitro* proliferation, plantlets.

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INTRODUCTION

Cassava is one of the crops in the tropical humid environments that is of great importance because of its availability as staple food, ease of cultivation, ability to change into different forms and stored as food for several years (Nassar *et al*., 2009; Le *et al*., 2007). It ranks fourth as a source of energy after rice, maize and sugarcane and it is a valuable source of calories especially in countries where malnutrition is widely spread (Chavez *et al*., 2000). It is predominantly grown by subsistence and commercial farmers in the sub-Saharan countries of Africa like Nigeria, Ghana, Burundi, Democratic Republic of Congo, Cameroon, Congo and host of others (Oyewole and Ogundele, 2001). Cassava improvement for increased production necessitates addressing various factors that beset its production. These factors include pests and diseases, genetics and breeding programs (Nassar and Ortiz, 2007) but more recently, it has been observed that micro-propagation has become an irreplaceable tool in the improvement and genetic manipulation of plant, especially vegetative propagated crops (Onuochi and Onwubiku, 2007). The ability to culture organized tissue in the form of very small shoots or meristem has allowed the most valuable application of plant tissue culture (Davies, 1981).

The techniques involved in Plant tissue culture were developed five decades ago (Santana *et al*., 2009) and since have been known to be powerful tools for studying and solving problems of production of cassava like many other crops

(Robert and Dennis, 2000; Adane, 2009). This technique is faster and requires less space than that necessary for conventional processes of preparing cassava cuttings (Loyola-Vargas and Vazques-Flota, 2006). It has also been proved through research findings that the techniques of plant tissue culture are the only realistic and efficient methods for supplying large number of true-to-type clean planting materials of any high value crop variety such as cassava within a short period of time.

Nevertheless, tissue multiplication needs the optimization of the plant hormones concentrations in the Murashige and Skoog (MS) medium in varying amounts with species since they determine the course of morphogenesis (Staden *et al*., 2008; IITA, 2009; Feyisola *et al*., 2015). Kane (2005) revealed Cytokinins, 6-Benzylaminopurine (BAP) and Kinetin (Kin) and Auxin, α-naphthalene-acetic acid (NAA) as the most widely used and effective (0.01-10 mg/L) hormones for shoot proliferation and root induction respectively. Smith *et al*., (1986) and Konan *et al*., (1997) were the first to report the successful production of an average of 5 to 6 shoots per bud from *in vitro* culture of cassava, though with low field survival rates (62-74%). Several workers have since these earlier studies also succeeded in the *in vitro* nodal culture of cassava (Medina *et al*., 2006; Escobar *et al*., 2009).

In Nigeria, *in vitro* mass propagation protocol for the production of cassava is still needed to ensure rapid mass propagation and dissemination of this crop to respond to the prevailing high interest of increasing its production for local consumption and exportation to other countries. In view of these, the aim of this study is todevelop an efficient *in vitro* mass propagation protocol for nodal cultures of cassava.

MATERIALS AND METHODS

Study location

The research was carried out in the tissue culture laboratory of the Nigeria Agricultural Quarantine Service (NAQS), Moore Plantation, Ibadan.

Source of cassava explants

The *in-vitro* plantlets of cassava used were obtained from the tissue culture laboratory of Genetic Resources Centre of the International Institute of Tropical Agriculture (IITA), Ibadan.

Nodal proliferation medium

Murashige and Skoog (MS) medium supplemented with higher levels of Cytokinin (BAP) and low Auxin (NAA) levels were prepared to culture the nodal explants of cassava as shown in Table 1. Nodal cuttings were aseptically dissected into 1-1.5cm long segments containing at least one node. The nodes were then cultured in the prepared MS media supplemented with various concentrations of hormones labelled treatments C_1, C_2, C_3 and C_4 (Table 1) for further proliferation of nodes with three replicates for each treatment.

The nodal explants were carefully cultured on the media and the culture tubes were sealed with Parafilm, labelled correctly and transferred to the culture room where they were kept and subjected to temperature range between 26^0C – 28^0C, 16 hours photo period (3,000-4,000 lux light intensity) supplied by florescent lamps. Node multiplication and plant height data were taken after three, six and nine weeks of culturing.

Data analysis

The average data estimated from the raw data taken were subjected to analysis of variance (ANOVA) using statistical analysis software (SAS), version 2.0 significant at $P<0.05$.

RESULTS

The mean of the treatments for the node multiplication medium and plantlets height using MS medium modified with hormones (Auxin and Cytokinin) on nodal culture of Cassava was presented in Tables 2 and 3 and graphically represented in Figures 1 and 2. Highest average number of nodes of 8.33 and 40.33 were recorded for the treatments C_1 (0NAA + 0BAP) and 7.67, 34.67 for C_3 (0.01NAA + 0.05BAP) at weeks 6 and 9 (Table 2) (Figure 1) (Plates 1a, b,c).

At 9 weeks the tallest plantlets height of 23.30 mm was observed in treatment C_3 (0.01NAA + 0.05BAP) while the lowest plant height was recorded for treatment C_2 (0.005NAA + 0.02BAP) with 2.87 mm (Table 3) (Figure 2).

Table 1: Various concentrations of Auxin and Cytokinin supplemented on Murashige and Skoog (MS) medium used for the *in-vitro* nodal proliferation of the Cassava plantlets.

Treatments	Hormone supplement (mg/l)
C_1	0NAA + 0BAP
C_2	0.005NAA + 0.02BAP
C_3	0.01NAA + 0.05BAP
C_4	0.015NAA + 0.08BAP

Table 2: Mean of nodes multiplication at different hormone concentrations.

Weeks	**Mean of treatments**			
	C_1 (mg/L) 0.0NAA+0.0BAP	C_2(mg/L) 0.005NAA+0.02BAP	C_3(mg/L) 0.01NAA+O.05BAP	C_4(mg/L) 0.015NAA+0.08BAP
3	1.33 ±1.2	2.33±1.2	2.67±0.6	2.67±1.2
6	8.33±2.5	3.67±0.6	7.67±2.5	3.67±0.6
9	40.33±1.5	6.33±2.1	34.67±15.0	8.00±3.6

Values are the Means ± S.D

Table 3: Average number of plantlets heights of Cassava on the different hormone concentrations.

Weeks	Plantlets height (mm)			
	C_1(mg/L) 0.0NAA+0.0BAP	C_2(mg/L) 0.005NAA+0.02BAP	C_3(mg/L) 0.01NAA+0.05BAP	C_4(mg/L) 0.015NAA+0.08BAP
Week 3	1.17±0.7	0.87±0.3	3.17±1.7	2.10±0.9
Week 6	6.00±1.1	1.67±0.7	6.27±1.5	3.00±1.2
Week 9	16.10±1.4	2.87±2.1	23.30±9.3	4.13±1.9

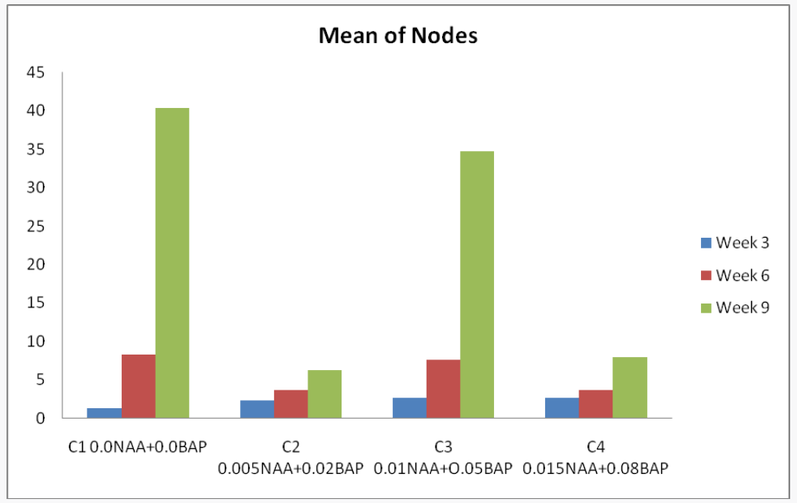


Figure 1: Mean of nodal proliferation rate of cassava at different growth regulators.

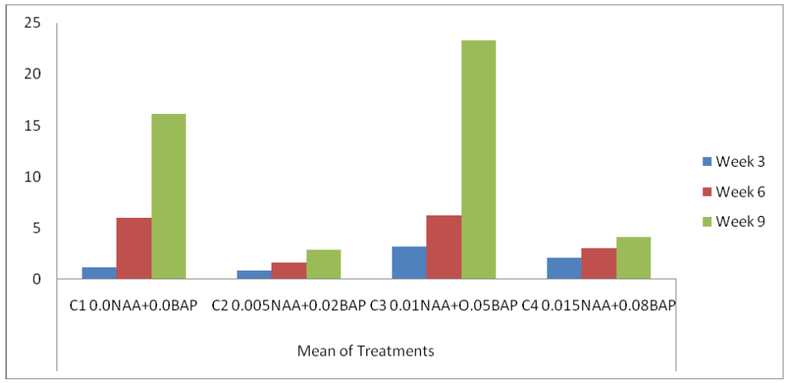
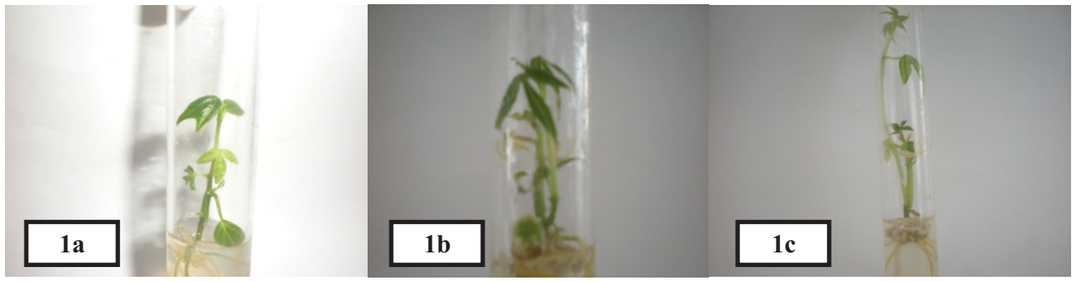


Figure 2: Means of the proliferated plantlets height of cassava at different concentration of hormones.



Plates 1a, b and c: Proliferated nodes and plantlets height at 3, 6 and 9 weeks of culture.

DISCUSSION AND CONCLUSION

It was observed from this study that *in vitro* nodal proliferation and development using nodal buds explants were best attained when the initiated plantlets were cultured onto full strength solid MS medium without any hormone supplement. This is in variance with Demeke *et al*. (2014) whose best result was obtained when BAP and Kinetin were used at the same concentrations. The shoot elongation was best when BAP concentration is a little higher than that of NAA. This suggested that Cassava shoot elongation before transplanting for acclimatization needs more of plant hormone Cytokinin than Auxin. This showed that the gene controlling height was stimulated not at early stage and a hormonal effect on plantlets was hereby affirmed in this study as justified by Esenowo (1999) and Onuochi and Onwubiku (2009). This study equally showed that Auxin and Cytokinin are good hormones at a concentration of 0.01mg/L of Naphthalene Acetic Acid and 0.05mg/L Benzyl Amino Purine (BAP).

However, there is need to further the research to obtain a concentration of the combination of both NAA and BAP that will support the nodal proliferation and equally with high plantlet height that would be better than control treatment of this study in which C_1 enhanced the nodal proliferation and have reduced height and C_3 with reduced nodal proliferation but increased plantlet multiplication performance.

In conclusion, the hormonal growth performance of *in-vitro* plantlet height of cassava tissue culture in this study revealed that plant hormones are necessary in the shoot elongation of cassava in tissue culture. The control experiment was a good hybrid that equally needs to be developed to know a hybrid that will carry a gene of desirable traits that can surpass the result from hormonal manipulations of cassava so that it would be cheaper and easily propagated on the field.

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