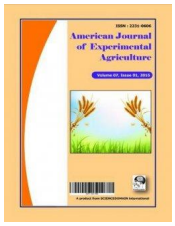

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In vitro Regeneration of Sweet Potato (*Ipomea batatas* (L.) Lam.) from Node Explants

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NCO and CII designed the study and wrote the protocol, while. Authors NCO and MUM performed the statistical analysis and wrote the first draft of the manuscript. All the authors read and approved the final manuscript.

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ABSTRACT

Investigations on *in vitro* clonal propagation of sweet potato (*Ipomea batatas* (L.) Lam.) were carried out at tissue culture laboratory of National Root Crops Research Institute, Umudike, Abia State, Nigeria. *Ipomea batatas* variety 440293 used was maintained in a culture room at 28 ±2°C under a 16 h photoperiod provided by white florescent tubes (60 µmo/lm-2s-1). Phytohormones- α-naphthaleneactic acid (NAA) at different concentrations and 6-benzylaminopurine (BAP) at 2 mg/l were used for the study. Summarily, data collected on the assessed growth parameters of the explants as tested with F test and t test showed that both the different levels of NAA and the number of weeks of application significantly (P< 0.05) enhanced *in vitro* production of sweet potato.

Keywords: Sweet potato; Ipomea batatas; in vitro regeneration.

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1. INTRODUCTION

The techniques of tissue culture are widely used in plant regeneration and transformation. *In vitro* vegetative propagation of crop or micropropagation is an important tool for the recovery, conservation of germplasm and embryo rescue [1]. It facilitates genetic manipulation of plants through somatic hybridization which previously were almost impossible, thereby has helped to overcome the limitations posed by basic or convectional breeding operations [2]. It is also effective in maintaining disease-free plants and avoiding genetic instability [3]. Plant tissue culture involves the isolation of cells, tissues and organs (otherwise called explants) from plants and growing them *in vitro* on a culture or nutrient media of known composition under a sterile controlled condition- an environment with optimized physical, nutritional and hormonal factors. [4] listed physical factors that must be maintained within certain limits for efficient *in vitro* vegetative propagation of crops to include temperature, pH, gaseous environment, light (quality and duration), and osmotic pressure. The culture vessel, nutrient or growth media, and the external environment (light, temperature etc) must meet both the physical and chemical needs of the *in vitro* cultured explants for optimal regeneration potential to be achieved.

In the regeneration of sweet potato, correct hormone combination ratio is one of the critical factors to consider in obtaining high regeneration efficiency [5,6]. Plant hormones or plant growth regulators (PGRs) in combination with other nitrogen sources make up the culture medium that supply all the essential mineral ions required for plant growth and development. Plant hormones have a profound effect on the morphology of the tissue developed from the explants as they can enhance not only the growth of some cultured slow growing tissues, but also determine the development pathway of the plant cells. In addition, it has been observed that plant hormones or pytohormones play an important role on the possibility for organogenesis or somatic embryogenesis to occur in culture [7], and the most frequently used PGRs in plant regeneration are auxins and cytokinins or their synthetic analogues. Studies have shown that the rate of proliferation of the root and shoot system are determined by the level of combination of auxins and cytokinins [8], also the type of culture established is determined by the ratio of auxins to cytokinins, and a correct phytohormone constitution ratio is necessary to optimize regeneration of plants [9]. Hence, the need to investigate the effect of combination of different concentrations of α-naphthaleneactic acid (NAA) at 2 mg/l of 6-benzylaminopurine (BAP) application, for optimal regeneration of sweet potato.

2. MATERIALS AND METHODS

Explants were excised from vigorously growing *in vitro Ipomea batatas* cultivar 440293 obtained from the *in vitro* gene bank housed at the Biotechnology programme (Plant tissue culture laboratory) of National Root Crops Research Institute (NRCRI) Umudike, Abia State, Nigeria.

Culture medium containing [10] was used for the study. Sucrose (30 g/l), myo-inositol (100 mg/l), and agar-E (7 g/l) were added to the culture medium.

The first MS medium- the control (CO) contained all the basal medium composition listed above only. The second MS medium contained the control and 6-benzylaminopurine (BAP) at a concentration of 2 mg/l. In third medium, α-naphthaleneactic acid (NAA) was added to the MS basal medium at different concentrations while the concentration of BAP was kept constant at 2 mg/l. The pH was adjusted to 5.8. Agar was dissolved in the three media by heating. 10ml each of the different media were dispensed into 150×25 mm test tubes and autoclaved at 121^{o}C at 1.05kg/cm^{2} for 15 minutes.

Explants were seeded singly onto medium in test tubes. The conditions of cultivation were at a temperature of 28±2^{o}C and artificial illumination supplied by white fluorescent tubes that provided an intensity of 60 µmol/m^{-2}s$^{-1.}$ The photoperiod was 16h of light and 8 h of darkness.

The design of the experiment was Completely Randomized Design (CRD). Eight treatments- six levels of NAA ranging from 0.05 to 3.0 mg/l, BAP at 2.0 mg/l, and the control that did contain neither NAA nor BAP- were replicated 25 times. Multivariate analysis of variance (MANOVA) was used to analyze the data collected on number of developed nodes, leaves, roots and plant height, between 1 to 5 weeks after subculture. Specifically the data was tested with F test and Ducan's t test using SPSS package 15.0.

3. RESULTS AND DISCUSSION

The F test result as displayed on Table 1 below shows that there were significant differences on the growth of *I. batatas* as measured by nodes, height, leaves, and roots, with respect to the different levels of NAA applied and the weeks of application at the $\alpha = 0.05$ level of significance.

The result of the Ducan's t test carried out to determine the particular level(s) of NAA and weeks of application that were actually responsible for the observed significant differences on the growth of *I. batatas* are shown in Tables 2 to 5 below. In Table 2, the result on the effect of different levels of NAA application on number of nodes shows that only two levels of NAA- 0.25 and 0.05 were statistically different from one another, while on plant height, only 0.05 level of NAA application was statistically different from all others.

On number of leaves, only 0.30 and 0.05 levels of NAA application were significantly different from each other, the result on number of roots clearly shows that the control and 0.10 NAA level were statistically different from each and all others. Furthermore, 0.30 level of NAA application was statistically different from 0.20, 0.10, and the control (Table 3).

The result on the effect of number of weeks of application of different levels of NAA on the number of developed nodes and plant height shows that significant differences exist among the weeks. No week was statistically the same with another. Again, the means were increasing as the weeks of application increases (see Table 4 below).

Table 5 below shows that the number of weeks of application of NAA significantly ($P<0.05$) improved number of developed leaves and roots. On number of leaves, while weeks 1, 2, 5 were statistically different from one another, week 3 was also not the same with weeks 1, 2 and 5. The f test result on number of roots shows that weeks 1, 4 and 5 were significantly different from each other.

Table 1. F test result on levels of NAA and number of weeks

Source	Dependent variable	Type III sum of squares	df	Mean squares	F	Sig.
NAA levels.	No of nodes	25.610	7	3.659	2.761	.036
	Plant height	50.785	7	7.255	9.832	.000
	No of leaves	68.903	7	9.843	2.933	.032
	No of roots	30.319	7	4.331	7.726	.000
No of Wks	No of nodes	815.103	4	203.776	153.771	.000
	Plant height	497.960	4	124.490	168.708	.000
	No of leaves	540.112	4	135.028	40.239	.000
	No of roots		4	8.232	14.684	.000

Table 2. Ducan's t test result on the effect of different levels NAA applied on number of nodes and plant height

NAA conc.	N	Number of nodes			Plant height		
		Subsets			Subsets		
		1	2	3	1	2	3
0.25	8	5.7120			4.5420		
0.30	8	6.6560	6.6560		5.0760	5.0760	
control	8	6.8680	6.8680		5.2540	5.2540	
0.20	8	6.8880	6.8880			5.7160	
0.10	8	6.9100	6.9100			6.1000	
0.15	8		7.6320	7.6320		6.2180	
0.05	8			8.6320			7.8080
Sig		.175	.267	.206	.192	.051	1.000

Note: mean values in the same column are statistically the same.
N represents sample size.

Table 3. Ducan's t test result on the effect of different levels of NAA applied on number of leaves and roots

Number of leaves				NAA levels	Number of roots				
NAA levels		Subsets			Subsets				
	N	1	2		N	1	2	3	4
0.30	8	5.2320		Control	5	3.1280			
0.25	8	5.6800		0.10	5		4.0960		
0.20	8	7.2320	7.2320	0.20	5			5.0000	
0.10	8	7.3520	7.3520	0.05	5			5.1520	5.1520
Control	8	7.6240	7.6240	0.25	5			5.5240	5.5240
0.15	8		7.9860	0.15	5			5.7560	5.7560
0.05	8		9.2000	0.30	5				5.9440
Sig.		.071	.135	Sig.		1.000	1.000	.083	.069

Note: mean values in the same column are statistically the same.
N represents the sample size

Table 4. Ducan's t test result on the effect of number of weeks of NAA application on number of developed nodes and plant height

weeks		Number of nodes					Plant height				
		Subsets					Subsets				
	N	1	2	3	4	5	1	2	3	4	5
Week1	5	1.2914					1.0157				
Week 2	5		3.9243					3.5000			
Week 3	5			6.8171					5.6357		
Week 4	5				8.4771					8.0514	
Week 5	5					14.7029					10.8786
Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Note: mean values in the same column are statistically the same.
N represents sample size

Table 5. Ducan's t test result on the effect of number of weeks of NAA application on number of developed leaves and number of roots

Weeks	N	Number of leaves				Number of roots		
		Subsets				Subsets		
		1	2	3	4	1	2	3
Week1	5	2.0471				4.1257		
Week 2	5		4.8514			4.4229		
Week 3	5			7.6343		4.6114		
Week 4	5			9.2057			5.2914	
Week 5	5				12.1943			6.2629
Sig.		1.000	1.000	.117	1.000	.170	1.000	1.000

Note: mean values in the same column are statistically the same
N represents sample size

In order to improve sweet potato through tissue culture, a reliable and efficient *in vitro* culture technique method that will ensure efficient differentiation- the development of the root and shoot system and whole plant regeneration is essential [11,12,13]. To achieve this, the ratio of the auxins to cytokinins (pytohormones) in the culture medium that supply all the essential mineral ions required for growth and development of *in vitro* cultured sweet potato has to be balanced so as to achieve an optimum culture medium that best supports *in vitro* regeneration of sweet potato. The combination of different levels of NAA at 2 mg/l of BAP, and the number of weeks of application (Tables 1-5) as to achieve an optimum culture medium that will benefit high regeneration efficiency significantly improved the *in vitro* production of sweet potato.

Apparently from the f test result as displayed on Tables 2 and 3, significant differences were observed on the growth parameters measured, with respect to different levels of NAA applied at 2 mg/l of BAP. The application of lower levels or concentration of NAA -0.5 to 0.15 mg/l enhanced the development of nodes, plant height and leaves, and had means that were significantly different unlike higher levels of NAA application. In fact, beyond 0.15 mg/l of NAA application further increase in the quantity of NAA applied did not statistically improve the growth of *Ipomea batatas* variety 440293. This result suggests that *Ipomea batatas* variety 440293 had appreciable quantity of endogenous NAA, hence it was only lower concentration of exogenously applied NAA that statistical improved its growth. The findings of this study is in line with that of [8], who observed that lower levels of auxins supported growth of taller plantlets in *in vitro* propagation of *Dioscorea rotundata* L. (White Yam).

The result on the number of weeks of application of the plant growth regulators on *in vitro* performance of *I. batatas* showed also significant differences on the growth parameters studied (Tables 4-5). Consistently the mean values of number of developed nodes and plant height increased as the number of weeks of application increases (Table 4), and were significantly different between one week and another. At week 5 the mean values of the number of developed nodes and plant height were still increasing considerably, suggesting that the optimum number of weeks of application may not have been attained. Almost a similar result was observed for number of developed leaves and roots (Table 5). On number of developed leaves, weeks 1, 2, and 5 were significantly different from each other, it was only weeks 3 and 4 that were statistically the same. On the other hand, the mean values on number of developed roots from week 1 to 3 were statistically the same. Significant differences were observed between weeks 4 and 5. Again, the mean values on both the number of developed leaves and roots were on the increase as the number of weeks of application increases, suggesting that the number of weeks of application is not adequate to enhance high regeneration efficiency. This is in line with the work of [14].

4. CONCLUSION

Phytohormones like NAA and BAP can enhance the *in vitro* propagation of Sweet potato. However, lower levels or concentration of NAA had more significant effect in the development of roots, leaves, nodes and plant height in sweet potato.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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