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# Biological activities of Naphthaleneacetic acid (NAA) and Benzylaminopurine (BAP) in *in vitro* production of sweet potato (*Ipomea batatas* (L.) Lam.).

1.

A LABORATORY EXPERIMENT TO DETERMINE THE APPROPRIATE CONCENTRATION OF NAA FOR *IN VITRO* SWEET POTATO PRODUCTION WAS CARRIED OUT AT THE NATIONAL ROOT CROP RESEARCH INSTITUTE (NRCRI) UMUDIKE. *IPOMEA BATATAS* VARIETY 440293 THAT WAS USED IN THIS STUDY WAS INCUBATED IN A CULTURE ROOM AT 28$^0$C AND EXPOSED TO ARTIFICIAL ILLUMINATION OF 2000-2500 *LUX* FOR 16 HOURS DAILY. THE EXPERIMENTAL DESIGN WAS COMPLETE RANDOMIZED DESIGN (CRD) WITH 8 TREATMENT AND 25 REPLICATES. THE RESULT SHOWED THAT EXOGENOUSLY APPLIED NAA AND BAP SIGNIFICANTLY IMPROVED THE *IN VITRO* PERFORMANCE OF *I. BATATAS.* THIS IS EVIDENT ON THE FACT THAT GENERALLY NONE OF THE TREATMENT AT DIFFERENT CONCENTRATION FORMED CALLUS. NAA PROMOTED THE HIGHEST MEAN NUMBER OF HEIGHT AND DEVELOPED LEAVES AT 0.05MG/L CONCENTRATION, AND DEVELOPED NODES AND ROOTS AT 0.15MG/L CONCENTRATION.

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## 1. INTRODUCTION

Plant hormones are the most critical component of plant tissue culture media. These organic compounds whether endogenous or synthesized, determine the relative growth and development of all organs in the plant. In fact *in vitro* culture is often impossible without these phytohormones (mainly auxins and cytokinins). They are active and have very significant effect even when present in very small quantities. However, their applications in vitro completely depend on the plant organ and species, as some plants are able to naturally synthesize sufficient growth hormones they need. Consequently do not need extra plant growth regulators to be added to the medium for *in vitro* growth and development (Pierik, 1987; Hangarter *et al*, 1980; Hussey and Stacey, 1981; Badoni and Chauhan, 2010).

Naturally occurring auxins like IAA are not very effective in supporting cultured plant tissues. They are easily destroyed by many tissues and unstable in the light; broken down by light in nutrient media and therefore must be stored in the dark. In addition they are also easily broken down by enzymes like peroxidases. When stored for a long period of time they gradually become inactive in aqueous solution. They are weak unlike the synthesized auxins like IBA (indole-3- butyric acid), 2,4-D (2, 4-dichlorophenoxyacetic acid) and NAA (1-naphthaleneacetic acid). Commercially available auxins are stable, effective and valuable. In fact they are widely used in agriculture for various purposes- in the prevention of premature dropping and thinning of fruits from stems, as rooting agents, synthetic herbicides, in micropropagation of various plants from vegetative parts like stem and leaf cutting etc.

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The various reports of workers on tissue culture studies shows that root and shoot development depend on auxin-cytokinin ratio. Organ differentiation in plants is regulated by changing the relative concentrations of these two growth hormones in tissue culture medium. Wang *et al* (1982) reported the regeneration of cassava via organogenesis in an auxin - cytokinin enriched medium containing NAA and BA. Also shoot regeneration procedure has been achieved via organogenesis from stem explant in a medium supplemented with NAA only (Gong *et al*., 2005). In a study on carrot, Ammirato (1985) found NAA to be more effective in contrast to other auxins in both initiation and maturation of somatic embryos. Hence, this study was set up to investigate the effect of different concentration of NAA and BAP in *in vitro* sweet potato production.

## 2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

The plantlets used in this experiment were from the explants of *Ipomea batatas* variety 440293 obtained from the *in vitro* gene bank of the Tissue Culture Laboratory of the National Root Crop Research Institute (N.R.C.R.I) Umudike, Abia State, were the study was also carried out. The nutrient media used contained macro and micro salts according to Murashige and Skoog (1962), iron salts, vitamins, myo-inositol, sucrose and phytagel or gelrite. In addition, NAA was added to the medium at different concentrations while BAP concentration was constant at 2mg/l in the experiment. The basal medium (that is the control) did contain neither NAA nor BAP.

Preparation of the NAA stock used was carried out by dissolving 10 mg of NAA in few drops of 0.5 N NaOH. After which distilled water was added to make up the solution up to 100 mL. Similarly, the BAP stock solution used in this study was prepared by dissolving 10 mg of BAP in 95% ethanol, and distilled water was added to make it up to 100 mL.

The medium was prepared by dissolving the various components of murashige and skoog (MS) medium. This freshly prepared medium was dispensed into the culture vessels and autoclaved at 121°C at 1.5 kg $cm^{-1}$ (15 psi) pressure for 15 minutes after adjusting its pH to 5.8 by few drops of 0.5N NOAH. Inoculated into this medium were very young healthy explants cut into two nodal parts. The explants were first washed in running water to reduce microbial load before transferring it into a beaker containing tween 20 placed in the laminar air flow hood already sterilized with 70% ethanol. Inorder to remove traces of foam they were washed severally in $DDH_2O$ after which the explants were transferred into a beaker containing 70% alcohol. The explants were placed in another beaker containing 0.5% NaOCl solution and stirred for 20 min after washing off the alcohol. Finally they were transferred into $DDH_2O$ and washed severally (at least 3 changes) till all traces of the sterilants were off. It was then sealed and labeled appropriately and kept inside the culture room maintained at 29°C±2°C and 16/8 duration photoperiod (see Plate 1).

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Plate 1: Sweet potato cultures arranged in the culture room.

The design of the experiment was Completely Randomized Design (CRD) and the total number of treatment combination was 8 with 25 replications to give a total of 200 experimental units.

The NAA levels were designated as $a_1$, $a_2$, $a_3$, $a_4$, $a_5$ and $a_6$, representing 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 all in mg/l. While the BAP at "0" was 2mg/l in the experiment, Basal medium "CO" did not contain NAA or BAP.

Data on the following growth parameter were collected on the 2, 4, 6, 8 and 10, weeks after culture- number of developed roots, nodes, leaves and height of explants. The data collected was analysed using analysis of variance (Anova).

## 3. RESULTS AND DISCUSSION

Mean number of developed nodes of *I. batatas*;

The result on the number of developed nodes is shown in the Table 1 below. The highest mean number of developed nodes of 17.40 cm was recorded at 0.15mg/l NAA concentration at week 10, while the lowest mean number of developed nodes 0.12 cm was recorded at 0.30mg/l NAA concentration at week 2. Generally the result obtained showed significant differences (P>0.05) at various level of concentration in all the weeks.

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Table 1: Mean number of developed nodes of *I. batatas*

| NAA/ TREATMENT | 2Wks | 4Wks | 6Wks | 8Wks | 10Wks |
|---|---|---|---|---|---|
| CO | 3.16 | 5.12 | 7.00 | 6.74 | 11.88 |
| BAP | 1.52 | 5.24 | 8.04 | 8.60 | 14.52 |
| 0.05 | 2.24 | 5.28 | 8.00 | 10.88 | 16.76 |
| 0.10 | 1.20 | 4.35 | 7.64 | 8.60 | 12.76 |
| 0.15 | 0.48 | 3.92 | 7.24 | 9.12 | 17.40 |
| 0.20 | 0.88 | 3.44 | 6.52 | 8.40 | 15.20 |
| 0.25 | 0.52 | 2.24 | 4.48 | 6.80 | 14.52 |
| 0.30 | 0.12 | 3.12 | 6.84 | 8.80 | 14.40 |
| LSD | 0.98 | 1.69 | 2.57 | 2.91 | 4.36 |

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Mean plant height of *I. batatas*:

Table 2 below shows the result on mean height of *I. batatas* as recorded in 2, 4, 6, 8, 10 weeks after subculture. The highest mean height value of 13.30 cm was recorded at week 10 at 0.05mg/l NAA concentration, while the lowest mean value of 0.00 cm was recorded at week 2 at 0.25mg/l NAA concentration. The result showed significant difference (P>0.05) in all the weeks, except at week 6 where the effect of the treatment was insignificant.

Table 2: Result on mean plant height of *I. batatas*

| NAA/ TREATMENT | 2Wks | 4Wks | 6Wks | 8Wks | 10Wks |
|---|---|---|---|---|---|
| CO | 2.16 | 3.71 | 5.40 | 6.15 | 8.85 |
| BAP | 0.94 | 5.52 | 8.97 | 10.28 | 13.30 |
| 0.05 | 1.68 | 5.66 | 7.34 | 11.06 | 13.30 |
| 0.10 | 1.34 | 3.96 | 6.29 | 8.51 | 10.40 |
| 0.15 | 0.76 | 3.91 | 6.60 | 8.57 | 11.27 |
| 0.20 | 0.86 | 3.19 | 5.21 | 7.84 | 11.48 |
| 0.25 | 0.00 | 1.96 | 4.28 | 6.24 | 10.23 |
| 0.30 | 0.31 | 2.11 | 4.33 | 7.99 | 10.64 |
| LSD | 0.77 | 2.02 | ns | 2.46 | 2.52 |

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Mean number of leaves of *I. batatas;*

The mean number of developed leaves is shown in the Table 3 below. The highest mean number of leaves of 15.60 was recorded at 0.05mg/l NAA concentration at week 10 while the lowest mean number of leaves of 0.76 was recorded at 0.30mg/l NAA concentration at week 6. The treatments showed significant difference (P>0.05) in all the weeks.

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Table 3: Mean number of leaves of *I. batatas*

| NAA/ TREATMENT | 2Wks | 4Wks | 6Wks | 8Wks | 10Wks |
|---|---|---|---|---|---|
| CO | 4.16 | 6.16 | 8.00 | 7.76 | 12.04 |
| BAP | 2.32 | 6.24 | 9.04 | 9.60 | 17.64 |
| 0.05 | 3.24 | 6.36 | 8.92 | 11.88 | 15.60 |
| 0.10 | 2.00 | 5.16 | 7.48 | 9.52 | 12.60 |
| 0.15 | 1.61 | 4.76 | 8.60 | 9.68 | 15.28 |
| 0.20 | 1.48 | 4.40 | 7.40 | 8.28 | 14.60 |
| 0.25 | 1.08 | 3.08 | 5.40 | 7.60 | 11.24 |
| 0.30 | 0.76 | 4.04 | 7.64 | 9.72 | 14.00 |
| LSD | 1.18 | 1.86 | 2.45 | 3.22 | 4.17 |

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Mean number of developed roots of *I. batatas;*

Mean number of developed roots taken at two weeks interval for ten weeks is displayed in Table 4 below. The highest mean number of developed roots of 8.24 was recorded at 0.15mg/l NAA concentration at week 6, while the lowest mean number of root of 3.44 at 0.10mg/l NAA concentration was observed at week 2. The result still recorded significant difference in all the weeks.

Table 4: Mean number of developed roots of *I. batatas*.

| NAA/ TREATMENT | 2Wks | 4Wks | 6Wks | 8Wks | 10Wks |
|---|---|---|---|---|---|
| CO | 2.88 | 2.96 | 3.08 | 3.52 | 3.20 |
| BAP | 3.16 | 5.04 | 8.76 | 3.84 | 4.28 |
| 0.05 | 4.92 | 5.24 | 6.68 | 4.60 | 4.32 |
| 0.10 | 3.60 | 4.64 | 5.12 | 3.44 | 3.68 |
| 0.15 | 4.68 | 6.56 | 8.24 | 5.00 | 4.32 |
| 0.20 | 3.52 | 5.24 | 6.60 | 4.96 | 4.68 |
| 0.25 | 4.08 | 5.76 | 6.68 | 5.56 | 5.56 |
| 0.30 | 5.20 | 6.68 | 7.44 | 5.20 | 5.20 |
| LSD | 1.81 | 2.12 | 2.77 | 5.20 | 1.58 |

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Discussion:

Among other important factors, a successful *in vitro* culture depends on the correct constituents and balance of growth regulators. The combination of phytohormones determines the nature of growth of cultured plant tissues. Investigations on the combination of plant hormones- auxins and cytokinins to enhance the

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performance of crop plant tissues in *in vitro* culture have long been recognized among researches (Pierik, 1987; Mantell, 2002; Cortes-Monllor and Liu, 1993; Ezeibekwe *et al*., 2009). In fact it is as an emerging scientific breakthrough that has enabled scientist to overcome limitations posed by basic breeding operations (Thottapilly *et al*., 1992; Mantell, 2000).

In this study, it was generally revealed that the exogenously applied synthetic auxin (Napthaleneacetic acid) and cytokinin (Benzylaminopurine) significantly improved the *in vitro* performance of sweet potato. The data collected on all the parameters studied: number of developed nodes, height, leaves and roots at the various weeks of subculture 2, 4, 6, 8 and 10, showed that hormonal application of NAA and BAP stimulated the growth of *I. batatas* variety 440293 as shown in Tables 1 – 4. This result also implies that the endogenous content of auxin and cytokinin in sweet potato variety 440293 was considerably low, hence the combination of the two hormones complemented each other enhancing the *in vitro* performance of *I. batatas* variety 440293. The result of this study is in line with works of Wang *et al.*, (1999) and Ezeibekwe *et al*., (2009).

On the mean number of nodes, it was observed that the highest mean number of nodes of 17.40 was recorded at 0.15mg/l NAA concentration on the 10$^{th}$ week (Table 1). This result also revealed that increase in the concentration of NAA beyond 0.15mg/l NAA concentration, inhibited node development of *I. batatas*. Hence, inferring that this concentration of NAA is best for node development in *in vitro* propagation of *I. batatas*. Similarly the result observed on root development as displayed in Table 4 shows that 0.15mg/l NAA concentration gave the highest mean value of 8.24 cm on root development on the 6$^{th}$ week.

The highest mean plant height of 13.0cm was recorded at 0.05mg/l NAA concentration on the 10$^{th}$ week, as shown in Table 2. Further increase in NAA concentration beyond 0.05mg/l, inhibited height development of *I. batatas*. Similarly, the result on the mean number of developed leaves as displayed in Table 3 shows that at 0.05mg/l NAA concentration, the highest mean number of developed leaves of 15.60 was also recorded. Further increase in concentration of NAA did not improve leave development in *I. batatas*, but rather inhibited it, inferring that 0.05mg/l is the optimum concentration for height and leave development in *I. batatas* variety 440293. This result is in agreement with the findings of Costa (2002).

It can be observed from this study that two levels of NAA concentration of 0.05mg/l and 0.15mg/l gave best result in all parameters studied. Lower level concentration of NAA (0.05mg/l) enhanced the development of height (highest mean height of 13.0 cm) and leaves (highest mean number of developed leaves of 15.60) while nodes and roots performed better at higher concentration of NAA (0.15mg/l). Ezeibekwe *et al*., (2009) observed that lower concentration of auxins produced taller plantlets. A similar observation was also reported by Gonzalez *et al*., (1999).

## 4. CONCLUSION

Exogenously applied NAA and BAP enhanced ~~leave,~~ height, nodes and root development in sweet potato. Generally lower concentration of NAA gave better result in all the parameters studied.

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