MICROPROPAGATION OF COCOYAM (*Xanthosoma sagittifolium*) USING DIFFERENT LEVELS OF BENZYLAMINOPURINE (BAP)

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Abstract

The experiment to determine the effect different levels benzylaminopurine on micropropagation of cocoyam (*Xanthosoma sagittifolium*) was carried out at the Tissue Culture Laboratory of the National Root Crop Research Institute (NRCRI), Umudike, Abia State, Nigeria. Two varieties of *Xanthosoma sagittifolium* ("ede uhie" and "ede ocha") used in the tissue culture room at 28 ^{0}C ± 2 and were exposed to artificial illumination of 2000 – 2500 lux for 16 hours daily. The experiment was a 2x6 factorial in a completely randomized design (CRD) with five replicates and the data collected was analyzed with Genstat Release VS 2008. Data were collected on plant height (cm), number of leaves, number of regenerated plantlets and fresh weight of regenerated plants. Significant differences were observed among the parameters studied; plant height, number of leaves, number of regenerated plantlets and fresh weight of regenerated plants, BAP application rather than improving the performances of the two cocoyam varieties had an inhibitory effect. The control (zero BAP application) recorded the best result with significant difference ($p<0.05$) from other BAP levels. Although fresh weight of regenerated plants appreciated at 0.05mgl^{-1} and 1.00mgl^{-1} BAP concentration for "ede uhie" and "ede ocha", respectively yet it was not significantly different. Generally "ede uhie" had a better performance in all the parameters studied.

Keywords: Micropropagation, Benzylaminopurine, Cocoyam, Artificial illumination, Regenerated Plants.

Introduction

Cocoyam improvement for increased production necessitates addressing various factors that beset its production, which include weeds, pests and diseases, requirement of large quantities of planting materials (which unfortunately is mostly part of the edible portion) for its propagation, low multiplication ratio and lack of improved planting materials which is the major constraint in cocoyam production (Dahniya and Kellon, 1983; Okigbo, 1986; Orkwor *et al*, in press). Although, plant breeding and genetics has contributed immensely in the improvement of crop plants, the improvement of cocoyam through hybridization is difficult due to flower production in the crop is irregular. In addition seed sett and seed germination is occasional or rare. However, micropropagation has been described as an irreplaceable tool in the improvement and genetic manipulation of plants especially vegetative propagated crops (Onwubiko and Mbanaso, 2006).

This new biotechnology technique involves the isolation of cells, tissues, organs etc from plants and growing them in a controlled condition *in vitro* termed tissue culture. This tool has been reported to be significant in the production of large number of plants or clones within a short time. It has also the ability to propagate plants species which are difficult to propagate vegetatively. Furthermore, it is capable of creating genetic variability and producing cocoyam corm with novel characteristics which could be more favourable than existing cocoyam varieties (Anura, 2006).

The establishment of culture media containing growth hormones or regulators and adjustment of their concentrations are keys to success in micopropagation. Individual growth regulators can be used alone or in combination with others to induce callus, root or shoot elongation or to develop shoots or somatic embryo into whole plants (Trigiano and Grey, 1996; Danso *et al*., 1999; Joseph *et al*., 1999; Ihemere, 2003). Benzylaminopurine (BAP) is one of the most successful plant regulatory synthetic cytokinin widely used for agricultural and horticultural experiments. This cytokinin has been to accelerate plant growth and cell division, increase size and multiple budding characteristics in a variety of tropical and sub-tropical fruits and vegetables, increase stem thickness, leaf surface and the

number of side branches. In fact the use of single treatment plant regulator in controlling some morphogenic developmental stages in crops have been reported (Triagiano and Gray, 1996; Matand *et al*., 2004). Thus the research was aimed at determining the effect of different concentrations of (BAP) in the micropropagation of cocoyam.

Materials and Method

This study was carried out at the Tissue Culture Laboratory of National Root Crop Research Institute (NRCRI) Umudike, Abia State, Nigeria. Umudike is located on latitude $5^{0}25^{1}$N, Longitude $7^{0}35^{1}$ and at 122m above sea level. The two cocoyam plantlets ("Ede ocha" and "Ede uhie") used for this study were collected from the tissue room in the tissue culture laboratory of NRCR1 Umudike and the nutrient media (BAP) contained inorganic and organic constituents. Six different levels of BAP concentrations (0, 0.25, 0.50, 0.75, 1.0 and 1.25MgL^{-1}) were used for the study.

The explants used were cut off from the mother plant (which is the planted corm piece) with knife, washed thoroughly and sterilized. Seventy percent ethanol and sodium hypochlorite were added to the explants in the bottle to remove impurities after which distilled water was used to rinse for about five minutes. The explants were properly cleaned and put in a 7ml of medium for culturing. The cultured two cocoyam varieties were brought out on Petri dishes using sterile forceps and the tissues subdivided into one shoot using sterile scalpel. These shoot cuttings were transferred into fresh media of different Benzylaminopurine concentrations. The neck of the culture vessels were flamed before replacing the cover. All these operations were carried out under strict aseptic conditions in the laminar airflow cabinet. The culture vessels were carefully labeled and incubated in the culture room 28 ^{0}C + 2 where they received artificial illumination of 2000-2500 lux for sixteen hours daily.

The experiment was laid out in a 2x6 factorial in completely randomized design (CRD) with 5 replicates. Data were collected on plant height (cm), number of developed leaves, number of regenerated plantlets and fresh weight of regenerated plantlets at 2 weeks interval for 6 weeks. With electronic weighing balance, fresh weight of the plantlets was determined after removal of plantlets from culture vessels on the 6th week. The data collected were statistically analyzed using Genstat Rlease, VS 2008.

Results

The effect of BAP concentration on plant height in the two cocoyam varieties is displayed on Table 1 below. At 2, 4 and 6 weeks. The different five levels of BAP application did not influence plant height, rather in the control there was significant difference ($p<0.05$) in plant height as the greatest mean of 3.92 and 4.20 was recorded for "ede ocha" and "ede uhie" respectively.

Table 1: Effect of different concentrations of BAP on plant height (cm) at 2, 4 and 6 weeks after planting (WAP)

Cocoyam Varieties	BAP Concentration (mgl^{-1})	Plant height		
		Time (weeks)		
		2	4	6
Ede ocha (E_1)	0.00	1.92	2.92	3.92
	0.25	1.54	1.57	2.54
	0.50	1.39	1.59	1.87
	0.75	1.28	1.51	1.77
	1.00	1.49	1.29	1.86
	1.25	1.06	1.08	1.20
$LSD_{0.05}$		**0.21**	**0.44**	**0.16**
Ede uhie (E_2)	0.00	2.19	2.39	4.20
	0.25	1.82	1.86	2.95
	0.50	1.52	1.65	2.39
	0.75	1.46	1.52	1.93
	1.00	1.14	1.26	1.83
	1.25	1.02	1.19	1.56
$LSD_{0.05}$		**0.07**	**0.11**	**0.11**

On the number of cocoyam leaves at 2, 4, and 6 weeks, the result was observed to be similar to that on plant height. Increase or decrease in BAP concentration did not yield any significant result in leaf formation. However, at 0.00 mgl^{-1} BAP concentration, the highest number of leaves was recorded in the two cocoyam varieties at 6 weeks (Table 2).

Table 2: Effects of different concentrations of BAP on number of leaves

Cocoyam Varieties	BAP Concentration (mgl^{-1})	Number of leaves		
		Time (weeks)		
		2	4	6
Ede ocha (E_1)	0.00	2.40	3.40	4.00
	0.25	1.80	2.60	2.80
	0.50	1.60	2.80	3.00
	0.75	1.40	2.00	3.40
	1.00	1.20	1.40	2.00
	1.25	1.00	1.20	1.40
$LSD_{0.05}$		**0.71**	**0.61**	**0.48**
Ede uhie (E_2)	0.00	2.60	3.00	3.80
	0.25	2.20	2.40	3.20
	0.50	1.80	2.20	3.00
	0.75	1.60	2.00	2.60
	1.00	2.00	2.80	3.00
	1.25	1.40	2.60	2.80
$LSD_{0.05}$		**0.61**	**0.53**	**0.50**

The result on the number of regenerated plantlets was slightly different from that on plant height and number of leaves. At 6 weeks. Generally, the highest number of regenerated plantlets was observed at 6 weeks at 0.00 mgl^{-1} BAP concentration in the two cocoyam varieties studied as shown in Table 3.

Table 3: Effects of different concentrations on number of regenerated plantlets

Cocoyam Varieties	BAP Concentration (mgl^{-1})	Number of regenerated plantlets		
		Time (weeks)		
		2	4	6
Ede ocha (E_1)	0.00	2.80	3.20	3.60
	0.25	2.20	2.60	3.40
	0.50	2.00	2.40	3.20
	0.75	2.20	2.80	3.00
	1.00	2.40	3.00	3.40
	1.25	2.40	2.60	2.80
$LSD_{0.05}$		**0.58**	**0.61**	**0.61**
Ede uhie (E_2)	0.00	3.00	3.60	3.80
	0.25	2.80	3.20	3.40
	0.50	2.60	2.80	3.00
	0.75	2.40	3.40	3.60
	1.00	2.80	3.00	3.20
	1.25	2.20	2.60	2.80
$LSD_{0.05}$		**0.58**	**0.61**	**0.58**

Similar to the result observed on the number of cocoyam leaves, increase in BAP concentration did not influence the fresh weight of the cocoyam varieties. However, the highest weight was recorded at 0.050 mgl^{-1} BAP concentration for "Ede ocha" although it was not significantly different ($p>0.05$) with the result from the control and other levels of BAP concentration (Table 4).

Table 4: Effect of different concentrations of BAP on fresh weight of regenerated plantlets

Cocoyam Varieties	BAP Concentration (mgl^{-1})	Fresh weight after 2 weeks
Ede ocha (E_1)	0.00	0.22
	0.25	0.16
	0.50	0.16
	0.75	0.08
	1.00	0.28
	1.25	0.15
$LSD_{0.05}$		**0.06**
Ede uhie (E_2)	0.00	0.18
	0.25	0.13
	0.50	0.38
	0.75	0.22
	1.00	0.20
	1.25	0.24
$LSD_{0.05}$		**0.06**

Discussion

The multiplication of many clonally propagated species in a short time is among many unique advantages of micropropagation systems (Penell, 1984). However, this was not achieved in the study on the effect of *in vitro* application of different concentration of synthetic cytokines (Benzylaminopurine) on the performance of two cocoyam varieties. In all the parameters investigated, the control (no BAP application) had better performances (Tables 1 to 4) inferring that the endogenous levels of BAP in the two cocoyam varieties were adequate. Hence the exogenous application of BAP did not have any positive effect on the performance of the crop rather had a considerable inhibitory role, and this is consistent with the physiological behaviour of hormones which have two concentrations, maximum for promotive and inhibitory effects. Berrie (1984) reported that synthetic cytokinins are inhibitory at high concentrations. Furthermore, it has been reported that the response of a plant to any particular exogenous applied hormone is influenced by a variety of other factors in the internal environment of the plant, chief among which is other hormones. Hence exogenous application of plant growth regulators may act by altering endogenous hormone systems, or by disrupting portions of the plant (Breece, 1987; Curtis *et al*; 1985). Invariably the effect of the plant growth hormone will not be expressed as expected. Again this may account for the result observed in this study.

Comparatively "Ede ocha" had a better performance than "Ede uhie" in all the parameters studied. Curtis *et al* (1985) had observed that the response to a particular hormonal message does not only depend on its current but also upon how it is interrupted by its recipient. Hence the reason for the variation in the performance of "ede uhie" and "ede ocha" to various levels of Benzylaminopurine concentration.

Generally, *in vitro* recalcitrance in growth parameters has been reported at BAP concentration of $1.25mgl^{-1}$ which is in agreement with the investigation on *in vitro* propagation of cassava; that above 1.0um BAP, shoot lengths were decreased which made subculture of nodes more difficult (Smith et al 1986 in Onuoha, 2006). Also, Anura (2006) observed that higher levels of kinetin (cytokinin) induced meristem cultures to form callus.

Conclusions

The application of plant growth regulators in crop improvement has a great potential and can offer meaningful results. However, this was not observed in the micropropagation of cocoyam using different levels of Benzylaminopurine, rather the application of BAP inhibited cocoyam production. It was the basel medium (no hormone) culture that appears to be the best practical and effective method for in vitro cocoyam propagation. Therefore, it is hereby recommended that other growth hormones like auxin, gibberellin be substituted for BAP in the micro propagation of cocoyam plantlets.

Acknowledgement.

We are grateful to Dr. (Mrs.) Mbanaso; the Head of the Department of Tissue Culture National Root Crop Research Institute (N.R.C.R.I), Umudike, Abia State and her staff for allowing the experiment to be conducted in their laboratory and for the provision some useful resources during the period of the research.

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