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SMicropropagation of Cassava (*Manihot esculantum* Crantz) Using Different Concentrations of Benzyaminiopurine (BAP)

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Abstract: An investigation conducted on the effect of Benzylaminopurine (BAP) on cassava plantlets cultures. Murashige and skoog medium containing different concentrations of BAP was used to subculture two varieties of cassava plantlets; TMS 98/0379 and TMS 98/0581, which were thereafter incubated in a culture room at 28°C ±2 and exposed to artificial illumination of 2000 -2500 lux for 16 h daily. Results showed that BAP application had some inhibitory effects on the two cassava varieties as only the control treatment (no BAP application) recorded the best growth in most of the growth parameters observed (height, leaves, nodes), and thus differing significantly ($p<0.05$) from other BAP levels. Between the experimental units, TMS 98/0379 appeared to grow better in terms of height, leaf and number of nodes while TMS 98/0581 recorded the greatest fresh weight.

Key words: Micropropagation, concentrations, BAP, cassava varieties, Nigeria

INTRODUCTION

Cassava improvement for increased production necessitates addressing various factors that beset its production, which include pests and diseases, requirement of large quantities of painting material for its propagation and low multiplication ratio (Dahniya and Kallon, 1983; Okigbo, 1986). Although plant breeding and genetics has contributed immensely in the improvement of crop plants, it has been observed more recently that micropropagation has become an irreplaceable tool in the improvement and genetic manipulation of plants especially vegetatively propagated crops (Onwubiko and Mbanaso, 2006). And forturnately cassava is ranked fourth in order of importance after rice, wheat and maize in crops for micropropagation in Africa (Johan *et al*., 1998).

The establishment of culture media and adjustment of their concentrations are the keys to success in micropropagation. Generally, cassava researchers have used specific treatment to obtain good development (Guohua, 1998; Joseph *et al*., 1999; Peng *et al*., 2001; Matand *et al*., 1994). Also studies an other crops have reported controlling some Morphogenic developmental stages using a single treatment (Triagiano and Gray, 1996; Matand *et al*., 1994). This study therefore, is an investigation on the effect of various levels of Benzylaminopurine (BAP) composition of culture media interacted with two cassava varieties for regeneration of cassava plants from stem explants.

MATERIALS AND METHODS

This study was carried out in the tissue culture laboratory of National Root crop Research Istitute (NRCRI) Umudike, Abia State Nigeria. Umudike is located at latitude 5° 25'N, longitude 7° 35' and at 122m above sea level. The two cassava plantlets (TMS 98/0379 and TMS 98/0581) used for this study were collected from the tissue room in the tissue culture laboratory of NRCRI Umudike and the nutrient media (BAP) contained inorganic and organic constituents. Six different levels of BAP concentrations (0, 0.25, 0.5,0. 75,1.0 and 1.25 Mg L^{-1}) were used for the study.

The two cassava varieties used were five months old plantlets cultured on a basal medium with height above 5 cm. The Plantlets were brought out on Petri dishes using sterile forcepts and the tissues subdivided into one-node length sterile scalpel. The node cuttings were transferred into fresh media of different BAP concentrations. The necks of the culture vessels were flamed before replacing

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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 at 28°C ±2 where they received artificial illumination of 2000-2500 Lux for 16 h daily.

The experimental design used for this study was a 2×6 factorial in a Completely Randomized Design (CRD) with 5 replications.

Data collection on plant height, number of leaves, number of developed nodes were collected on 2 weeks interval for 6 Weeks After Subculture (WAS). With electronic weighing balance fresh weight of the plantlets was determined after removal of plantlets from culture vessels on the 6th week. The statistical analysis used in analyzing the result collected was genstat.

RESULTS

At 2,4 and 6 WAS, BAP application did not influence plant height in the two cassava varieties, rather in the control there was significant difference($p<0.05$) in plant height as the greatest mean (3.88) was recorded (Table 1). Again at BAP level 1.25 mgt^{-1} TMS 98/0581 recorded a high percentage of callus formation showing *in vitro* recalcitrance.

The result on number of cassava leaves at 2,4 and 6 WAS was almost similar to that of plant height. Increase or decrease in BAP concentration was not consistent with leaf formation in the two cassava varieties. However, at 0.75 mg L^{-1} BAP, TMS 98/0581 recorded the highest number of leaves at 6 WAS (Table 2) although this was not significantly ($p<0.05$) greater than the leaf formation in the control.

A significant difference in the number of nodes was however observed in 2,4 and 6 WAS between the two cassava varieties (Table 3). Apparently, TMS 98/0379 had

Table 1: Effect of different concentration of BAP on plant height at 2,4and 6 WAS

Treatments	Weeks after subculture		
Cassava varieties	2	4	6
TMS 98/0379(c_1)	1.30	1.72	2.12
TMS 98/03819 (c_2)	1.04	1.72	1.12
LSD (0.05)	0.28	1.19	1.47
LSD (0.05)			
BAP Mgl $^{-1}$			
0.00 (B_0)	1.78	2.58	3.88
0.25 (B_1)	1.26	1.47	1.53
0.50 (B_2)	1.24	1.46	1.72
0.75 (B_3)	1.10	1.49	1.67
1.00 (B_4)	1.79	0.80	0.99
LSD 0.05	0.48	0.57	0.64

Table 2: Effect of different concentration of BAP on number of leaves at 2,4and 6 WAS

Treatments	Weeks after subculture		
Cassava varieties	2	4	6
TMS 98/0379(c_1)	1.65	2.84	2.87
TMS 98/03819 (c_2)	0.95	2.26	2.78
LSD (0.05)	0.46	0.80	0.91
LSD (0.05)			
BAP Mgl $^{-1}$			
0.00 (B_0)	2.20	3.70	4.40
0.25 (B_1)	1.32	2.47	2.14
0.50 (B_2)	1.02	2.95	3.43
0.75 (B_3)	1.18	2.82	3.43
1.00 (B_4)	1.00	2.00	2.00
LSD 0.05	1.07	1.35	1.54
LSD (0.05)	0.80	1.39	1.58

Table 3: Effect of different concentration of BAP on number of nodes at 2,4and 6 WAS

Treatments	Weeks after subculture		
Cassava varieties	2	4	6
TMS 98/0379(c_1)	3.04	3.75	4.74
TMS 98/03819 (c_2)	1.60	2.64	3.75
LSD (0.05)	0.47	0.73	1.00
LSD (0.05)			
BAP Mgl $^{-1}$	2.50	4.50	6.10
0.00 (B_0)	2.88	3.36	4.22
0.25 (B_1)	2.48	3.61	4.71
0.50 (B_2)	2.34	3.46	4.33
0.75 (B_3)	2.10	2.30	3.30
1.00 (B_4)	1.63	1.94	2.82
LSD 0.05	0.81	1.27	1.74

Table 4: Effect of different concentration of BAP on number of nodes at 2, 4 and 6 WAS

Treatments	6 Weeks after subculture
Cassava varieties	
TMS 98/0379(c_1)	0.18
TMS 98/03819 (c_2)	1.23
LSD (0.05)	2.06
LSD (0.05)	
BAP Mgl $^{-1}$	0.25
0.00 (B_0)	0.14
0.25 (B_1)	0.19
0.50 (B_2)	0.11
0.75 (B_3)	0.42
1.00 (B_4)	0.13
LSD 0.05	3.56

number of no des was observed at 4 and 6 WAS at 0.00MgL^{-1} BAP concentration, although this was not significantly different from other hormonal levels.

Similar to the result observed in the number of cassava leaf, the flesh weight of the two cassava varieties of constants increase or decrease in BAP concentration (Table 4). The highest fresh weight was recorded for the two cassava varieties at 1.0 MgL^{-1} BAP concentration although it was not significantly different ($p>005$) with the result from the control and other levels of BAP. Comparatively TMS 98/0581 recorded a greater fresh weight.

DISCUSSION

The *in vitro* application of different concentration of synthetic cytokinins (BAP) considerably inhiabited the performance of the two cassava varieties used in the study. The fact that all the parameters evaluated were better in the control treatment (no BAP application) shows that the endogenous levels of BAP in these cassava varieties were adequate. Hence, exogenous application of BAP led to supra-optimal amounts which induced some inhibitory effects. This observation is consistent with the physiological behaviour of hormones which have two concentration maxima for promotive and inhibitory effects. Berrie (1984) reported that synthetic cytokinins are inhibitory at high concentration. Again, the effect of any particular exogenously applied growth hormone is influenced by a variety of other factors in the internal environment of the plant, especially other hormones in the plant (Cutis and Barnes, 1985; Preece, 1987).

Comparatively, TMS 98/0379 appeared to have performed better in terms of height, leaves, and nodes. This is in agreement with the findings of Curtis and Barnes (1985) who reported that the response to a particular hormonal message does not only depend on its content but also upon how it is "read" by its recipient. The *in vitro* recalcitrance observed in TMS 98/0581 at BAP concentration 1.25 MgL^{-1} is also in agreement with the findings on *in vitro* propagation of cassava; that above 1.0 μm BAP, shoot lengths were decreased which made subculture of nodes more difficult. Also, Anura (2006) observed that higher levels of kinetin (Cytokinin) induced meristem cultures to form callus.

CONCLUSION

Apparently, from the forgoing, the use of plant growth regulators like BAP has great potential and can offer meaningful and useful results. However, cassava cultures using basal media (no hormone) appears to be the most practical and effective method for maximum *in vitro* cassava performance.

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