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Effects of Combination of Different Levels of Auxin (NAA) and Cytokinin (BAP) on *in vitro* Propagation of *Dioscorea rotundata* L. (White Yam)

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Abstract: Studies were carried out with the aim of evaluating *in vitro* the effects of growth regulators-auxin (NAA) and cytokinin (BAP) combined at different levels on *Dioscorea rotundata* regeneration potentials on modified Murashige and Skoog media. Concentrations of 0, 0.25, 0.5, 0.75, 1.00 mg L^{-1} and 0, 0.1, 0.2, 0.3 and 0.4 mg L^{-1} of NAA and BAP, respectively were used to subculture healthy white yam plantlets. The plant height, number of leaves, nodes, vines, roots and fresh weights were evaluated. Results obtained when analyzed at 5% level of significance showed that the concentration of both hormones (auxin and cytokinin) had significant effects on plant regeneration. BAP (0.2 mg L^{-1}) in combination with NAA (0.5 mg L^{-1}) showed more increase in almost all the parameters measured when compared to other concentrations combined.

Key words: Effects, different combinations, auxin and cytokinin, *in vitro*, propagation, *dioscorea rotundata*

INTRODUCTION

The white guinea yam, *Dioscorea rotundata* is a monocotyledon, native of the rainforest zones of West Africa (Onwueme, 1998) and belongs to the edible species of Dioscoreaceae. Among the species making up the yams, the white guinea yam is the most widely cultivated. The yield is low, probably because not much reaearch efforts have been dedicated to the genetic improvement of this specie (Onwueme, 1994). Yams are however difficult to breed by hybridization because of their polyploidy and high heterozygosity. So far, the quickest means of crop plant multiplication has been through *in vitro* micro propagation. According to Otto *et al.* (2005), the minisett technique has gone a long way towards solving the problem in the yam belt of West Africa.

Plant tissue culture carried out under aseptic conditions has important applications in plant biotechnology. The potential impact of emerging technologies such as micropropagation techniques via *in vitro* propagation may be assessed by their potential efficiency to overcome the limitations posed by basic breeding operations (Thottapilly *et al.*, 1992; Mantell, 2000).

The method of using tissue culture in the propagation of white yam is effective in maintaining disease-free plants and avoiding genetic instability (Long, 1989). Cortes-Monllor and Liu (1993) achieved four fold multiplication in *D. rotundata* within 2-3 months using a nodal segment of 0.5-1 cm grown on Murashige and Skoog (1962) medium with 2 mg L^{-1} IAA and 2 mg L^{-1} kinetin followed by transferring the shoot to MS medium with 1 mg L^{-1} Naphthalene Acetic Acid (NAA) to obtain roots and found that the age of the nodal segment influenced the growth. Node cuttings of *D. rotundata* and *D. alata* were also regenerated to plantlets on MS medium supplemented with 2% sucrose, 0.5 mg L^{-1} kinetin, 20 mg L^{-1} cytokinin and 0.6% agar (Ng, 1994, 1996). Though, the technique of tissue culture has been used for the propagation of white yam, yet much has not been done on the effects of various concentrations of BAP and NAA on the *in vitro* regeneration of complete plants of white yam, hence this research is aimed at helping to ascertain the best level of combination of the two phytohormone and also help to create a new yam cropping technique.

MATERIALS AND METHODS

This research was conducted at the tissue culture laboratory of National Root Crop Research Institute, Umudike, Umuahia, Abia State, Nigeria in September, 2008. The yam plantlets used were obtained from the culture room of the Institute. The nutrient media contained minerals and elements according to Murashige and Skoog (1962).

The NAA stock solution was prepared by dissolving 10 mg of NAA in few drops of 0.5 N NaOH. Distilled water was added to make up the solution up to 100 mL. The BAP stock solution was prepared by dissolving 10 mg of BAP in 95% ethanol and made up to 100 mL using distilled

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water. The 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 and BAP were added to the MS medium in different concentrations and combinations (Table 1).

The pH of the medium was adjusted to 5.8 with drop wise of 0.5 N NaOH. It was then dispensed into culture vessels and autoclaved at 121°C at 1.5 kg cm^{-1} (15 psi) pressure for 15 min.

Healthy explants were isolated from initiated mother plants in the culture room. Explants were washed in running water to reduce microbial load. They were later transferred into a beaker containing tween 20 placed in the laminar air flow hood already sterilized with 70% ethanol. They were washed several times with DDH_2O till all traces of foams were off. They were transferred into a beaker containing 70% ethanol and stirred for 1 min.

The alcohol was washed off and they were transferred into another beaker containing 0.5% NaOCl solution and stirred for 20 min. Finally they were transferred into DDH_2O and washed severally (at least 3 changes) till all traces of the sterillants were off. Each explant was cut into two nodal parts and inoculated into the freshly prepared culture media. It was then sealed and labeled appropriately and kept inside the culture room maintained at 29°C±2°C and 16/8 duration photoperiod. These were observed daily for growths. Readings were taken after 8, 10 and 12 weeks after subculture.

Table 1: Effects of combination of different levels of Auxin (NAA) and Cytokinin (BAP) on the number of vines of *in vitro* propagation of *Dioscorea rotundata*

	Weeks after subculture		
Treatments (Mg L^{-1})	8	10	12
BAP (0) + NAA (0)	2.20^{def}	2.80^{ef}	3.40^{bc}
BAP (0) + NAA (0.25)	2.20^{def}	3.00^{ef}	3.60^{bc}
BAP (0) + NAA (0.5)	2.40^{def}	3.20^{def}	3.20^{bc}
BAP (0) + NAA (0.75)	2.60^{def}	2.80^{ef}	3.20^{bc}
BAP (0) + NAA (1.0)	3.20^{cdef}	3.40^{cdef}	3.60^{bc}
BAP (0.1) + NAA (0)	2.20^{def}	3.00^{ef}	3.60^{bc}
BAP (0.1) + NAA (0.25)	2.20^{def}	3.00^{ef}	3.80^{bc}
BAP (0.1) + NAA (0.5)	2.0^{def}	3.0^{ef}	3.80^{bc}
BAP (0.1) + NAA (0.75)	4.40^{bcde}	4.50^{bcdef}	4.00^{bc}
BAP (0.1) + NAA (1.0)	1.20^{f}	2.00^{f}	2.20^{c}
BAP (0.2) + NAA (0)	4.20^{bcde}	4.60^{bcdef}	4.80^{bc}
BAP (0.2) + NAA (0.25)	5.00^{bc}	5.60^{bcde}	6.20^{b}
BAP (0.2) + NAA (0.5)	9.20^{a}	9.40^{a}	9.60^{a}
BAP (0.2) + NAA (0.75)	5.80^{bc}	6.00^{bcde}	6.20^{b}
BAP (0.2) + NAA (1.0)	6.20^{b}	6.50^{b}	6.30^{b}
BAP (0.3) + NAA (0)	6.00^{b}	6.40^{bc}	6.30^{b}
BAP (0.3) + NAA (0.25)	6.00^{b}	6.20^{bcd}	6.20^{b}
BAP (0.3) + NAA (0.5)	5.80^{bc}	6.00^{bcde}	6.10^{b}
BAP (0.3) + NAA (0.75)	6.20^{b}	6.50^{b}	6.30^{b}
BAP (0.3) + NAA (1.0)	6.20^{b}	6.40^{bc}	6.30^{b}
BAP (0.4) + NAA (0)	4.80^{bcd}	5.20^{bcde}	6.10^{b}
BAP (0.4) + NAA (0.25)	4.60^{bcde}	5.80^{bcde}	6.00^{b}
BAP (0.4) + NAA (0.5)	6.20^{b}	6.50^{b}	6.30^{b}
BAP (0.4) + NAA (0.75)	4.90^{bcd}	5.20^{bcde}	5.40^{bc}
BAP (0.4) + NAA (1.0)	5.60^{bc}	6.00^{bcde}	6.10^{b}
LSD (0.05)	2.70	3.08	3.21

Means with the same letter(s) down the column are not significantly different

RESULTS AND DISCUSSION

Hormonal application of BAP and NAA stimulated the production of vine in *D. rotundata*. BAP (0.2) + NAA (0.5) mg L^{-1} recorded the highest mean in this regard which differed significantly ($p<0.05$) from other combinations of NAA and BAP (Table 1). The same trend was also observed in number of leaves and nodes produced (Table 2, 3 and Fig. 1).

As regards to plant height, the control BAP (0) + NAA (0) produced taller plantlets with longer internodes than other levels. The heights of plantlets in the other media series were shorter and with reduced internodes especially when there was an increased BAP concentration in the medium. This differed significantly at ($p<0.05$) (Table 4 and Fig. 2).

From Table 5, BAP (0.2) + NAA (0.5) mg L^{-1} gave the highest mean value when the number of roots produced were analysed. It was noted that media series with 1.0 mg L^{-1} NAA concentration irrespective of the BAP concentrations moved toward callus formation of the plantlets (Table 5 and Fig. 3).

Table 2: Effects of combination of different levels of Auxin (NAA) and Cytokinin (BAP) on the number of leaves of *in vitro* propagation of *Dioscorea rotundata*

	Weeks after subculture		
Treatments (Mg L^{-1})	8	10	12
BAP (0) + NAA (0)	1.20^{f}	1.40^{h}	1.40^{d}
BAP (0) + NAA (0.25)	1.40^{f}	2.20^{gh}	2.40^{cd}
BAP (0) + NAA (0.5)	1.60^{f}	2.20^{gh}	3.00^{bcd}
BAP (0) + NAA (0.75)	1.60^{f}	2.20^{gh}	3.20^{bcd}
BAP (0) + NAA (1.0)	1.60^{f}	2.60^{fh}	3.2^{bcd}
BAP (0.1) + NAA (0)	1.80^{ef}	2.80^{efgh}	3.20^{bcd}
BAP (0.1) + NAA (0.25)	1.80^{ef}	3.20^{defgh}	3.80^{bcd}
BAP (0.1) + NAA (0.5)	2.00^{ef}	3.20^{defgh}	3.80^{bcd}
BAP (0.1) + NAA (0.75)	2.20^{def}	3.20^{defgh}	4.00^{bcd}
BAP (0.1) + NAA (1.0)	2.80^{cdef}	3.40^{cdefgh}	4.00^{bcd}
BAP (0.2) + NAA (0)	3.60^{cde}	3.80^{bcdefg}	3.50^{bcd}
BAP (0.2) + NAA (0.25)	3.60^{cde}	3.80^{bcdefg}	4.80^{bc}
BAP (0.2) + NAA (0.5)	6.80^{a}	7.80^{a}	8.40^{a}
BAP (0.2) + NAA (0.75)	4.80^{b}	5.60^{b}	5.40^{b}
BAP (0.2) + NAA (1.0)	4.60^{b}	5.50^{bc}	5.40^{b}
BAP (0.3) + NAA (0)	4.00^{bcd}	5.20^{bcd}	4.80^{bc}
BAP (0.3) + NAA (0.25)	4.00^{bcd}	4.80^{bcde}	4.40^{bc}
BAP (0.3) + NAA (0.5)	4.00^{bcd}	4.80^{bcde}	4.40^{bc}
BAP (0.3) + NAA (0.75)	4.00^{bcd}	4.40^{bcdef}	4.20^{bcd}
BAP (0.3) + NAA (1.0)	4.20^{bc}	4.20^{bcdefg}	4.20^{bcd}
BAP (0.4) + NAA (0)	4.50^{bc}	4.70^{bcdefg}	4.00^{bcd}
BAP (0.4) + NAA (0.25)	4.00^{bcd}	4.20^{bcdefg}	4.00^{bcd}
BAP (0.4) + NAA (0.5)	4.80^{b}	4.90^{bcde}	4.80^{bc}
BAP (0.4) + NAA (0.75)	4.00^{bcd}	5.00^{bcd}	5.20^{bc}
BAP (0.4) + NAA (1.0)	4.20^{bc}	4.40^{bcdef}	4.80^{bc}
LSD (0.05)	1.80	2.14	2.85

Means with the same letter (s) down the column are not significantly different

Table 3: Effects of combination of different levels of Auxin (NAA) and Cytokinin (BAP) on the number of nodes of *in vitro* propagation of *Dioscorea rotundata*

	Weeks after subculture		
Treatments (Mg L^{-1})	8	10	12
BAP (0) + NAA (0)	1.00^{e}	2.00^{fgh}	1.40^{f}
BAP (0) + NAA (0.25)	1.00^{e}	2.00^{fgh}	1.40^{f}
BAP (0) + NAA (0.5)	1.20^{de}	2.40^{efgh}	1.60^{ef}
BAP (0) + NAA (0.75)	1.00^{e}	2.60^{efgh}	1.60^{ef}
BAP (0) + NAA (1.0)	1.40^{cde}	2.66^{efgh}	1.60^{ef}
BAP (0.1) + NAA (0)	1.40^{cde}	2.60^{efgh}	1.60^{ef}
BAP (0.1) + NAA (0.25)	1.40^{cde}	2.78^{efgh}	1.80^{def}
BAP (0.1) + NAA (0.5)	1.40^{cde}	2.80^{efgh}	2.00^{cdef}
BAP (0.1) + NAA (0.75)	1.80^{abcde}	2.80^{efgh}	2.00^{cdef}
BAP (0.1) + NAA (1.0)	1.80^{abcde}	2.92^{efgh}	2.20^{bcdef}
BAP (0.2) + NAA (0)	1.60^{cde}	1.80^{gh}	2.20^{bcdef}
BAP (0.2) + NAA (0.25)	1.60^{cde}	3.00^{efgh}	2.40^{bcdef}
BAP (0.2) + NAA (0.5)	4.90^{a}	6.58^{a}	4.80^{a}
BAP (0.2) + NAA (0.75)	3.00^{bc}	4.80^{bc}	3.40^{b}
BAP (0.2) + NAA (1.0)	3.60^{b}	5.00^{b}	3.40^{b}
BAP (0.3) + NAA (0)	3.60^{b}	3.18^{cdefgh}	3.20^{bc}
BAP (0.3) + NAA (0.25)	2.80^{bcd}	3.40^{cdefg}	3.00^{bcd}
BAP (0.3) + NAA (0.5)	2.20^{cdef}	3.90^{bcdef}	3.00^{bcd}
BAP (0.3) + NAA (0.75)	2.40^{cde}	3.60^{bcdef}	3.20^{bc}
BAP (0.3) + NAA (1.0)	2.20^{cdef}	3.80^{bcdef}	3.20^{bc}
BAP (0.4) + NAA (0)	2.00^{cdefg}	4.00^{bcdef}	3.00^{bcd}
BAP (0.4) + NAA (0.25)	2.20^{cdef}	4.06^{bcd}	2.90^{bcde}
BAP (0.4) + NAA (0.5)	2.80^{bcd}	4.66^{bcd}	3.00^{bcd}
BAP (0.4) + NAA (0.75)	2.40^{cde}	4.72^{bcd}	3.40^{b}
BAP (0.4) + NAA (1.0)	2.40^{cde}	4.08^{bcde}	3.20^{bc}
LSD (0.05)	1.04	1.56	1.38

Means with the same letter(s) down the column are not significantly different.

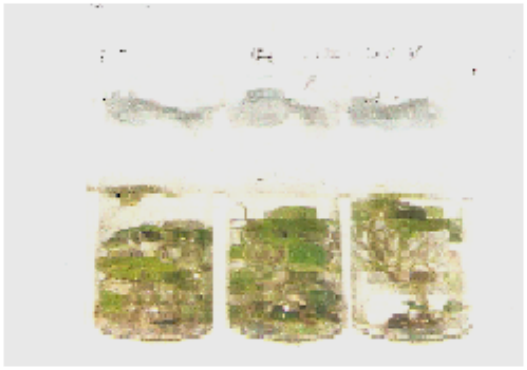

Fig. 1: Best growth at concentration BAP (0.2) + NAA (0.5) mg L^{-1}

The trend in the response of *D. rotundata* in terms of fresh weight was not consistent with either the increase or decrease in BAP and NAA combinations (Table 6). It was found that BAP (0) + NAA (0.5) mg L^{-1} recorded the highest mean value for fresh weight (Table 6). This however was not of any significance ($p>0.05$) when compared to BAP (0.2) + NAA (0.5) mg L^{-1} but differed from other levels of BAP and NAA combination.

Both phytohormones Auxin and Cytokinin are important in *in vitro* propagation, since combination of both favours the *in vitro* performance of *D. rotundata* to

Table 4: Effects of combination of different levels of Auxin (NAA) and Cytokinin (BAP) on Plant height of *in vitro* propagation of *Dioscorea rotundata*

	Weeks after subculture		
Treatments (Mg L^{-1})	8	10	12
BAP (0) + NAA (0)	11.92^{a}	11.95^{a}	12.00^{a}
BAP (0) + NAA (0.25)	7.00^{b}	7.02^{b}	7.50^{b}
BAP (0) + NAA (0.5)	5.40^{b}	5.48^{b}	6.50^{b}
BAP (0) + NAA (0.75)	4.70^{b}	4.80^{b}	6.08^{b}
BAP (0) + NAA (1.0)	4.68^{b}	4.72^{b}	5.60^{b}
BAP (0.1) + NAA (0)	4.60^{b}	4.66^{b}	4.98^{b}
BAP (0.1) + NAA (0.25)	4.56^{b}	4.50^{b}	4.98^{b}
BAP (0.1) + NAA (0.5)	4.28^{b}	4.46^{b}	4.78^{b}
BAP (0.1) + NAA (0.75)	4.28^{b}	4.42^{b}	4.74^{b}
BAP (0.1) + NAA (1.0)	4.12^{b}	4.38^{b}	4.62^{b}
BAP (0.2) + NAA (0)	4.00^{b}	4.36^{b}	4.58^{b}
BAP (0.2) + NAA (0.25)	3.64^{b}	4.36^{b}	4.52^{b}
BAP (0.2) + NAA (0.5)	3.64^{b}	4.06^{b}	4.50^{b}
BAP (0.2) + NAA (0.75)	3.52^{b}	3.94^{b}	4.46^{b}
BAP (0.2) + NAA (1.0)	3.40^{b}	3.82^{b}	4.22^{b}
BAP (0.3) + NAA (0)	3.32^{b}	3.80^{b}	3.82^{b}
BAP (0.3) + NAA (0.25)	3.30^{b}	3.38^{b}	3.80^{b}
BAP (0.3) + NAA (0.5)	3.20^{b}	3.20^{b}	3.38^{b}
BAP (0.3) + NAA (0.75)	3.14^{b}	3.18^{b}	3.32^{b}
BAP (0.3) + NAA (1.0)	3.06^{b}	3.14^{b}	3.22^{b}
BAP (0.4) + NAA (0)	3.02^{b}	3.04^{b}	3.20^{b}
BAP (0.4) + NAA (0.25)	3.02^{b}	2.92^{b}	3.20^{b}
BAP (0.4) + NAA (0.5)	3.00^{b}	2.78^{b}	3.14^{b}
BAP (0.4) + NAA (0.75)	2.52^{b}	2.66^{b}	3.10^{b}
BAP (0.4) + NAA (1.0)	2.50^{b}	2.40^{b}	2.50^{b}
LSD (0.05)	4.84	4.42	0.41

Means with the same letter(s) down the column are not significantly different.

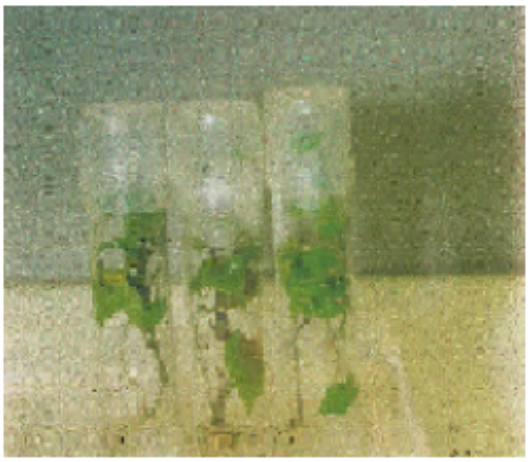

Fig. 2: Tall plantlets obtained at BAP (0) + NAA (0) mg L^{-1}

give an optimum result. Also the performance of all the parameters in BAP (0.2) + NAA (0.5) showed that the endogenous content of these two phytohormones are low in *D. rotundata*, hence their combination complement each other. Ammirato (2004) reported that in *D. bulbifera* and *D. alata*, cytokinin at moderate concentrations enhanced shoot development. Charturvedi (1977)

Table 5: Effects of combination of different levels of Auxin (NAA) and Cytokinin (BAP) on the number of roots of *in vitro* propagation of *Dioscorea rotundata*

	Weeks after subculture		
Treatments (Mg L^{-1})	8	10	12
BAP (0) + NAA (0)	2.40^{defghi}	3.00^{defg}	4.20^{defgh}
BAP (0) + NAA (0.25)	4.60^{cde}	5.20^{cde}	6.20^{cdef}
BAP (0) + NAA (0.5)	9.20^{b}	11.00^{b}	12.00^{b}
BAP (0) + NAA (0.75)	0.4^{ghi}	1.00^{g}	1.20^{i}
BAP (0) + NAA (1.0)	0.00^{I}	1.00^{g}	1.20^{i}
BAP (0.1) + NAA (0)	2.60^{defghi}	4.40^{cdefg}	$4.40^{cdefghi}$
BAP (0.1) + NAA (0.25)	5.20^{cd}	6.40^{cd}	7.80^{c}
BAP (0.1) + NAA (0.5)	5.60^{c}	6.80^{c}	7.40^{cd}
BAP (0.1) + NAA (0.75)	$2.80^{cdefghi}$	2.80^{defg}	3.00^{fghi}
BAP (0.1) + NAA (1.0)	1.20^{ghi}	1.40^{fg}	1.42^{hi}
BAP (0.2) + NAA (0)	0.00^{i}	1.00^{g}	1.40^{i}
BAP (0.2) + NAA (0.25)	4.20^{cdef}	5.40^{cde}	6.60^{cde}
BAP (0.2) + NAA (0.5)	12.30^{a}	15.20^{a}	15.60^{a}
BAP (0.2) + NAA (0.75)	4.20^{cdef}	6.20^{cd}	7.40^{cd}
BAP (0.2) + NAA (1.0)	1.40^{fghi}	1.50^{fg}	2.80^{fghi}
BAP (0.3) + NAA (0)	0.40^{ghi}	1.00^{g}	2.80^{fghi}
BAP (0.3) + NAA (0.25)	3.20^{cdefg}	$.4.80^{cdef}$	6.60^{cde}
BAP (0.3) + NAA (0.5)	3.00^{cdefgh}	4.60^{cdefg}	6.20^{cdef}
BAP (0.3) + NAA (0.75)	2.60^{defghi}	4.40^{cdefg}	5.20^{cdefg}
BAP (0.3) + NAA (1.0)	0.20^{hi}	1.80^{efg}	2.00^{ghi}
BAP (0.4) + NAA (0)	2.60^{defghi}	4.40^{cdefg}	4.20^{defghi}
BAP (0.4) + NAA (0.25)	$2.80^{cdefghi}$	4.20^{cdefg}	5.00^{defgh}
BAP (0.4) + NAA (0.5)	2.60^{defghi}	4.00^{cdefg}	5.00^{cdefgh}
BAP (0.4) + NAA (0.75)	2.20^{efghi}	3.00^{defg}	$4.60^{cdefghi}$
BAP (0.4) + NAA (1.0)	2.00^{efghi}	3.00^{defg}	3.80^{efghi}
LSD (0.05)	2.92	3.67	3.57

Means with the same letter(s) down the column are not significantly different

Fig. 3: Callused tissues obtained at concentration of BAP 0) + NAA (1.0) mg L^{-1}

obtained from cultures of *D. floribunda*, an average of 5-6 shoot in 20 days by culturing single node cuttings on a medium containing 8.8 μm BAP which is in agreement with this work. The control, BAP (0) + NAA (0) mg L^{-1} gave the tallest height which is also in agreement with Lakshmi *et al.* (2006) when they observed that the growth and morphogenetic responses of *in vitro* cultures depend among other factors on

Table 6: Effects of combination of different levels of Auxin (NAA) and Cytokinin (BAP) on fresh weight of *in vitro* propagation of *Dioscorea rotundata*

Treatments (Mg L^{-1})	12 weeks after subculture
BAP (0) + NAA (0)	0.12^{fg}
BAP (0) + NAA (0.25)	0.33^{bcd}
BAP (0) + NAA (0.5)	0.55^{a}
BAP (0) + NAA (0.75)	0.35^{bc}
BAP (0) + NAA (1.0)	0.32^{bcde}
BAP (0.1) + NAA (0)	0.28^{cdef}
BAP (0.1) + NAA (0.25)	0.26^{cdefg}
BAP (0.1) + NAA (0.5)	0.18^{cdefg}
BAP (0.1) + NAA (0.75)	0.24^{cdefg}
BAP (0.1) + NAA (1.0)	0.35^{bc}
BAP (0.2) + NAA (0)	0.33^{bcd}
BAP (0.2) + NAA (0.25)	0.27^{cdef}
BAP (0.2) + NAA (0.5)	0.50^{ab}
BAP (0.2) + NAA (0.75)	0.15^{defg}
BAP (0.2) + NAA (1.0)	0.32^{bcde}
BAP (0.3) + NAA (0)	0.20^{cdefg}
BAP (0.3) + NAA (0.25)	0.27^{cdef}
BAP (0.3) + NAA (0.5)	0.26^{cdef}
BAP (0.3) + NAA (0.75)	0.23^{cdef}
BAP (0.3) + NAA (1.0)	0.14^{efg}
BAP (0.4) + NAA (0)	0.17^{cdefg}
BAP (0.4) + NAA (0.25)	0.11^{fg}
BAP (0.4) + NAA (0.5)	0.26^{cdefg}
BAP (0.4) + NAA (0.75)	0.19^{cdefg}
BAP (0.4) + NAA (1.0)	0.08^{g}
LSD (0.05)	0.19

Means with the same letter(s) down the column are not significantly different

the correct constituents and balances of growth regulators. Synergy between BAP and NAA exhibited positive effect in the induction of roots. The phenomena of *in vitro* micro tuberization were observed in MS medium with 0.25 mg L^{-1} BAP and 0.5 mg L^{-1} NAA. This phenomenon was also observed by Mantell (2002) when he cultured *D. rotundata* with media containing 5 mg L^{-1} IAA and in the presence of 0.5 mg L^{-1} kinetin. The *in vitro* recalcitrance observed in the MS medium with 1.0 mg L^{-1} NAA irrespective of BAP levels is in agreement with the findings of Belarmino and Rosario (1991), who cultured axillary buds of yam, observed that above 1.0 mg L^{-1} of NAA, roots move towards callusing. Also Aslam *et al.* (2006) showed that a callogenic response varies from hormonal concentration whether applied singly or in combination.

The classic experiments of Skoog and Miller (1957) showed that at higher concentrations of phytohormones, a hard undifferentiated callus was obtained consisting of small thick walled cells.

CONCLUSION

In this study, it was noted that the control NAA (0 mg L^{-1}) + BAP (0 mg L^{-1}) produced taller plantlets than other levels. Also the heights in other media series produced plantlets with reduced heights and short internodes when the BAP level was increased. The MS

media containing 0.5 mg L^{-1} NAA + 0 mg L^{-1} BAP was optimal for production of higher fresh weight compared to other combinations.

RECOMMENDATION

The outcome of this research therefore recommend the use of different levels of combinations of phytohormones for *in vitro* propagation of *D. rotundata* for the genetic improvement of this specie.

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