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The Effect of the 2,4-Dichlorophenoxy Acetic Acid, Benzyl Adenine and Paclobutrazol, on Vegetative Tissue-Derived Somatic Embryogenesis in Turmeric (*Curcuma* var. Chattip)

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ABSTRACT

The nodal explants of Curcuma var. Chattip could develop callus after in vitro culturing and transplanting within 4 weeks in MS medium supplemented with 1.0 mg l^{-1} 2,4-D, although this optimal if the media was further supplemented with 5.0 mg l-1 BA, obtaining the highest number of new shoots in 6 weeks. MS medium supplemented with 0.01 mg l^{-1} paclobutrazol and 15% (v/v) coconut water was found suitable for regenerating the highest number of new shoots (7.25 shoots). The number of leaves per plantlet, length of leaves, and length of petrioles were significantly reduced ($p \leq 0.05$) when increased concentrations of paclobutrazol and especially paclobutrazol with 15 % (v/v) coconut water. However further experimentation is required to evaluate the dose-response and the interaction between coconut water and paclobutrazol. In contrast, there were no significant difference in leaf width in all treatments.

1. Introduction

Turmeric (*Curcuma* var. chattip), a herbaceous plant of the Zingiberaceae (ginger) family (Purseglove,1972), is an economically important cultivated species in Thailand being grown for cut flowers, decoration and landscaping. Although propagated by underground rhizomes,

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the rate of rhizome multiplication and growth is very low, making this non-viable for large scale economic production. Moreover, many diseases and pests, particularly soft rot caused by *Pythium* spp. (Jantan *et al.*, 2003) as well as bacterial wilts are consistently threatening *Curcuma* var. chattip. This problem is compounded by its slow propagation rate which seriously impedes on the ability to replace diseased plants quicker than infection rates.

To aid the rapid and large scale propagation of this plant, *in vitro* formation of storage organs such as rhizomes that can be directly transferred to the field without any acclimatization has been reported (Balachandran *et al*., 1990). However, further improvement of the protocol to obtain larger and more vigorous plantlets is required in order to approach an economically or logistically viable method.

The presented investigation was carried out to examine the effects of the plant growth regulators palcolbutrazol and benzyl adenine (BA) in the presence of napthaleneacetic acid (NAA) and coconut water, upon the formation of callus and multiply new plantlets.

2. Materials and Methods

Spouted immature shoots (ca. 1 cm. long) were collected and used as explants. They were washed thoroughly in running tap water and soaked with liquid detergent (teepol solution) followed by rinsing in tap water for 2 min. For surface sterilization, explants are rinsed in 10 % (v/v) Clorox solution for 10 min and 5 % (v/v) Clorox solution for 10 min and finally soaked with sterile distilled water three times to remove traces of Clorox. Immature shoots are trimmed to remove excess tissue. Murashige and Skoog medium (MS) fortified with 0.25 % (w/v) and 4% (w/v) final concentration of gelrite and sucrose, respectively. MS medium supplemented with 2,4-dichlorophenoxy acetic acid (2,4-D), BA, and coconut water is used as basal medium for callus. The pH of medium was adjusted to 5.6 by using 0.1 N NaOH or 0.1 HCl and autoclaving at 121 °C for 20 min to sterilize. All cultures were incubated under 16hours photoperiod (irradiance of 36 µmole m^{-2} S^{-1}) with temperature 25 ± 1°C. We observed samples at regular intervals and scored for callus and shoot growth, with 10 independent replicates being used for each experimental treatment.

3. Statistical Analysis

This experiment used CRD (Completely Randomized Design). The analysis of variance between the means was conducted by using Duncan's multiple range test (Duncan, 1955).

4. Result and Discussion

After 4 weeks of *in vitro* culture, MS medium with 1.0 mg l^{-1} 2,4-D and no BA showed a remarkable degree of callus formation. Indeed, the callus obtained from *in vitro* culture was faster growing, delicate, mostly spongy, and white creamy in color (Table 1). In addition, with 0.5 mg l^{-1} 2,4-D concentration, slightly more explants grew callus, and grew more callus per explants, when using MS medium supplemented with 0.5 mg l^{-1} and 1.0 mg l^{-1} BA. This studies is in broad agreement with Nayak (2000) and Hosoki andSagawa(1977) who reported the callus induction from leaf base as explants of *curcuma aromatica* using 1.5 mg l^{-1} 2,4-D and 1.0 mg l^{-1}BA. However, in this system it is yet to be evaluated if BA will enhance the highest level of callus production seen with 1.0 mg l^{-1} 2,4-D.

Table 1: The average number of explants induced to form callus (as 5%) and degree of callus per explants after culturing for 4 weeks.

MS medium plus	% of explants induced callus	Degree of Callus response
2,4-D 0.5 mg l^{-1}	53.4	a
2,4-D 1.0 mg l^{-1}	78.2	c
2,4-D 2.0 mg l^{-1}	56.7	a
2,4-D 0.5mg l^{-1}+BA 0.1mg l^{-1}	49.86	a
2,4-D 0.5 mg l^{-1}+BA 0.5mg l^{-1}	66.67	b
2,4-D 0.5 mg l^{-1}+BA 1.0mg l^{-1}	61.34	b

a – Slight callusing, b – more callusing, and c – Profuse callusing

Callus obtained from *in vitro* cultivation (Table 1) were used to investigate the influence of growth regulators on the induction of somatic embryogenesis.

Four-week old callus were used for multiplying new shoot by culturing them on MS basal medium supplemented with 0.1 mg l^{-1}NAA, 15% (v/v) coconut water and varying levels (viz.1.0, 2.0,3.0, 4.0, and 5.0 mg l^{-1}) of BA. All independent *in vitro* cultures suggested that the observed effect of BA was likely to be mediated via inducing new shoots, as summarized in Table 2. After six weeks, new shoots regenerated from callus. MS medium supplemented with 0.1 mg l^{-1},15% CW and 5.0 mg l^{-1} BA gave the highest average levels of new shoots attained per callus (5.1 ± 0.54) as worked of Dekker *et al*. (1991) did with ginger but Jala (2011) cultured *Curcuma longa* in MS medium supplemented with 2 mg l^{-1} BA.

Callus obtained from above mentioned medium are used to investigate the influence of the growth retardant (paclobutrazol) on the growth of somatic embryogenesis.

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After six weeks, callus was regenerated to new shoots on MS medium supplemented with 0.1 mg l^{-1} NAA and 5.0 mg l^{-1} BA. On this condition, callus could form somatic embryos and germinated (Table 2) within 6 weeks, allowing this system to investigate the effect of paclobutrazol.

Table 2: Number of new shoots regenerated from callus that are cultured on MS medium supplemented with 0.1 mg l^{-1} NAA 15% (v/v) coconut water with varied BA concentrations.

NAA (mg l^{-1})	CW(%)	BA(mg l^{-1})	Number of new shoots*
0.1	15	1.0	1.2 e
		2.0	1.8 d
		3.0	2.5 c
		4.0	3.2 b
		5.0	5.1 a

* Means within columns with different letters are significantly different by using DMRT at the ($p \leq 0.05$) level.

Table 3: The effect of paclobutrazol and coconut water on somatic embryo regeneration from callus within 8 weeks of *in vitro* culturing.

MS+NAA0.1mgl^{-1}, BA5.0mgl^{-1}, 4 %sucrose (control) plus	No. of multiple shoots*	No. of leaves per plantlet *	The leaf length (cm)*	The leaf width $(cm)_{ns}$	Length of leaf petriole (cm)*
Control (no Paclobutrazol)	4.00 c	7.82 a	3.80ab	0.73	5.53 a
Paclobutrazol 0.01 mg l^{-1}	3.00 d	7.67 a	3.65 b	0.84	4.11 b
Paclobutrazol 0. 1 mg l^{-1}	5.00 b	6.92 ab	3.37 bc	0.78	3.36 cd
Paclobutrazol 1.0 mg l^{-1}	4.00 bc	7.08 ab	3.53 b	0.86	3.97 bc
Paclobutrazol 5.0 mg l^{-1}	3.75 c	5.86 c	3.97 a	0.75	4.40 b
Paclobutrazol 10.0 mg l^{-1}	4.50bc	7.54 a	3.25 c	0.85	3.61 c
Paclobutrazol 0.01 mg l^{-1}+ cw15%	7.25 a	4.57 c	4.33 a	0.87	3.60 c
Paclobutrazol 0.1 mg l^{-1} + cw15%	3.75 c	5.48 c	3.66 b	0.82	3.70 c
Paclobutrazol 1.0 mg l^{-1} +cw15%	6.25 a	5.33 c	4.08 a	0.82	3.41 cd
Paclobutrazol 5.0 mg l^{-1} +cw15%	3.00 de	6.60 bc	3.93 a	0.79	2.51 de
Paclobutrazol 10.0 mgl^{-1} +cw15%	2.5 e	2.65d	2.15 d	0.91	e

*Means within columns with different letters are significantly different from each other ($p \leq 0.05$), ns - no significant difference for that trait across all culture conditions.

4.1 Effect of plant growth (retardant – paclobutrazol) on somatic embryo

The callus were used to investigate the influence of the growth retardant (paclobutrazol) on growth of somatic embryogenesis by cultured them in MS basal medium supplemented with 0.1 mg l^{-1} NAA, 5 mg l^{-1} BA, with and without 15% (v/v) coconut water (CW) and varied concentrations of paclobutrazol (viz. 0.01, 0.1, 1.0, 5.0 and 10 mg l^{-1}) for 8 weeks. During this time the number of multiple shoots, number of leaves, leaf length, leaf width, and length of each leaf petriole were measured as shown in Table 3.

Results indicated that paclobutrazol and 15% (v/v) CW both induced a significant difference ($p \leq 0.05$) on plantlet regeneration as measured by four out of five traits (Table 3). Only the average leaf width showed no statistically significant differences between the different treatments. However, length of leaf petriole and length of leaf were not significantly different. The numbers of leaves per plantlet were all broadly decreased by increasing paclobutrazol concentrations, especially in the presence of coconut water where the broadly similar effects at lower concentrations of paclobutrazol in the presence of 15% (v/v) coconut water.

However, with respect to the number of adventitious shoots the situation is less clear with no clear trend. In general, Paclobutrazol alone showed no clear if any concentration dependent effect upon the number of multiple shoots per explants but, the data for Paclobutrazol in the presence of 15% (v/v) coconut water is more erratic with the two highest average number of shoots per explants (7.25 shoots and 6.25 shoots for 0.01 and 1.0 mg/l Paclobutrazol, respectively) intermixed amongst lower values with the lowest attained with 10 mg/l Paclobutrazol. Direct somatic embryogenesis has been reported in many other plants (e.g. Nadguada *et al*.1978, Salvi *et al*. 2000, Shiqurkar *et al*. 2001, Yasuda *et al*. 1988).

5. Conclusion

Immature nodal explants of *Curcuma* var. Chattip could develop callus after *in vitro* culturing in MS medium supplemented with 1.0 mg l^{-1} 2,4-D within 4 weeks. Subculturing callus in MS basal medium supplemented with 0.1 mg l^{-1} NAA, 15% coconut water and 5.0 mg l^{-1} BA yielded the highest number of new shoots. These could be further induced somatic embryo regeneration and leaf formation by *in vitro* culturing in MS medium supplemented with 0.1 mgl^{-1} NAA, 5.0 mgl^{-1} BA, 15% (v/v) coconut water and (0.01 to10.0 mg l^{-1}) paclobutrazol. Although equivocal as complex interactions may be occurring, the data suggests that 0.01 and 1.0 mg l^{-1} paclobutrazol and 15% (v/v) CW were the most suitable conditions leaded to formation of the highest number of new shoots (7.25 and 6.25 shoots per explants, respectively) within 6 weeks.

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