Effects of NAA BA and Sucrose on Shoot Induction and Rapid Micropropagation by Trimming Shoot of Curcuma longa L.

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Abstract

Shoot tips of Curcuma longa L. were used as explants. These explants were cultured on MS medium supplemented with various concentrations of (0, 1, 2, 3 and 4 mg/l) BA and (0, 0.5, and 1 mg/l) NAA. They were significant difference ($p \leq 0.05$) in each parameter. Explants which were cultured on MS medium supplemented with 1 mg/l NAA and 2 or 3 mg/l BA gave the highest average number of new shoots (2.4, 2.6 shoots, respectively) and number of leaves (5.4 leaves), optimum number of roots (2.6 roots per shoot), and plant height (4.5 cm). MS medium supplemented with only 2 mg/l BA produced the highest average number of shoot (2.6 shoots) and 5.4 leaves per shoot. Explants were trimmed in longitudinal sections (LS) to 2 and 3 sections. At 2 sections gave the highest number of new shoots (4.3 shoots per section). Explants which were cultured on MS medium with 60 gm/l gave the highest average shoots and leaves per bunch, and longest and biggest size of root.

Keyword: Curcuma longa L.; BA; NAA; sucrose; trimming

1. Introduction

Curcuma longa L. belongs to Family Zingiberaceae, and produces edible rhizome which is used as a medicine and food additive. Rhizome is used for peptic ulcer [8], gastic ulcer [6], and dyspepsia [8], [21]. Micropropagation gave many plantlets which were true to type and provided uniform plants [5] . High quality *Curcuma longa,* with high and constant level of curcuminoids and volatile oil, as used to multiple plants. *In vitro* propagation of *Curcuma longa* has been done [1], [8], [10], [11], [16], and [21]. Plant tissue culture is one method that is used for micropropagation by culturing pieces of meristematic tissue on synthetic medium. This increass the number of plants in a short period [5]. Young shoots tip and lateral buds which are meristmatic tissue, are used as explants [18]. Trimming shoot tips for increasing surface area also which enhances new shoots.

This paper investigates the size of shoot tips which are trimmed in small pieces, and studies the effect of BA NAA and sucrose concentration, which affect the number of new shoots and new roots for large scale propagation .

2. Materials and Methods

MS medium was used as culturing medium. Sprouts of young shoot tips of about 1.5 cm long were used as explants. All explants were washed many times with running tap water, then soaked in 70% alcohol for 3 min and transferred to 10% clorox for 20 min, and followed by 5% Clorox for 10 min, then, rinsed 3 times with sterile distilled water for 3 min each. All shoot bud explants were cultured on MS medium supplemented with vary concentration of BA (0, 1, 2, 3, 4 mg/l) and NAA (0, 0.5, 1 mg/l) for initiation, elongation, regeneration, and shoot bud formation . The second experiment: Shoot tips of about 0.5 cm long were used for trimming in longitudinal section with half (2 sections) and half (3 sections) per shoot tip. The third experiment: Shoot tips of about 0.5 cm long were cultured on MS medium supplemented with 20, 30, 40, 50, 60 gm/l of sucrose. All cultures were placed at 25±2 ° C under cool white florescent light (37 μmolm-2 S-1) for 16/8 h photoperiod.

Statistical analysis

The data were subjected to one way analysis of variance (ANOVA) to assess treatment differences and interaction using the SPSS version 11.0 Significance between means was tested by DMRT's Test ($p \leq 0.05$). This experiment had 25 replications per treatment.

3. Result and Discussion

Cultured explants on MS medium were grown with vary concentration of BA (1, 2, 3, and 4 mg/l) and NAA (0, 0.5 and 1 mg/l) Clean cultures of explants were subcultured every 3 weeks in the same culture medium for 4 times. Explants grew up and the parameters of growth were studied.

3.1 Effect of NAA and BA on growth of plantlet

Shoot tips of about 0.5 cm were used as explants and cultured on MS medium of varied concentrations of BA (0, 1, 2, 3, and 4 mg/l) and NAA (0, 0.5 and 1 mg/l). All parameters, which are number of new shoots, leaves, roots, and plants height were recorded after culturing for 12 weeks. It was found that all parameters were significantly different ($p \leq 0.05$) among their treatments as shown in Table 1.

It is evident from Table 1 that among all treatments, the average number of 2.6 and 2.4 new shoots were the highest. These new shoots were cultured on MS medium supplemented with 1 mg/l NAA and 2 or 3 mg/l BA, (respectively) . MS medium supplemented with only 2 mg/l BA also produced the highest average number of new shoots (2.6 shoots), number of leaves (5.4 leaves), and optimum number of roots (2.6 roots per shoot), and plant height (4.5 cm). As [19] had done with *Curcuma angustifolia,* Rozbi used 3 mg/l BAP to produce 6.9 shoots per explant within 6 weeks. Reference [9] and [19] worked with *Curcuma longa* and cultured young shoot on WPM medium supplemented with 4 mg/l BAP, which was the best medium to regenerate new shoot (6.25 shoots per plant) within 2 weeks. *Zingiber officinale* Rosc. which is related to turmeric, was reported by [2]. Reference [7] and [20] reported that MS medium, containing 2.0 mg/l BAP and 1 mg/l NAA, was able to regenerate the optimum clonal propagation of turmeric by rhizome bud culture.

3.2 Induced new shoots from trimming shoot tips

After trimming shoot tips in longitudinal sections to 2 and 3 sections, all of the sections were cultured on MS medium supplemented with 3 mg/l BA. After culturing the section of shoot tips for 4 weeks, many new shoots were regenerated at the base of sections which contact the medium in each section, and the results are recorded in Table 2. The average number of new shoots which regenerated from each sections was significantly different ($p \leq 0.05$) from each other. Shoot tips which were trimmed in half (2 sections) gave the highest number of new shoots (4.3 shoots) when compared to the control (2.2 shoots), and trimmed in 3 sections (3.5 shoots), was the second.

Table 1 Average number of new shoots, roots, leavess and plant height of Curcuma longa L. on MS medium supplemented with a combination of BA and NAA after culturing for 12 weeks.

MS supplemented with		number of new shoots*	number of leaves*	number of roots*	plant height (cm)*
NAA (mg/l)	BA (mg/l)				
0	0	1.8bc	0.6f	0.6fg	2.34gh
0	1	1.2d	1.0ef	1.2def	3.04ef
0	2	2.6a	5.4a	2.6bc	4.5b
0	3	1.6cd	0.6f	1.6de	3.34def
0	4	1.8bc	3.2b	1.8de	4.78a
0.5	0	1.0d	2.2c	2.2cd	4.86a
0.5	1	1.6cd	1.2ef	0.6gh	3.46cde
0.5	2	1.2d	0.0	0.2h	2.28gh
0.5	3	1.0d	1.8d	0.6gh	3.56bcd
0.5	4	1.6cd	2.0c	1.6de	3.14def
1.0	0	1.0d	0.6f	0.8fg	3.00ef
1.0	1	1.4cd	2.2c	2.8b	3.12def
1.0	2	2.4ab	1.6de	3.0ab	3.6bcd
1.0	3	2.6a	1.6de	2.6bc	3.44cde
1.0	4	1.8bc	4.0ab	3.2a	3.36def

* significant difference ($p \leq 0.05$),
a-h Average compared mean within a column by Duncan's multiple range test ($p \leq 0.05$)

Table 2 Average of new shoots per section of Curcuma longa, which were cultured on MS medium supplemented with 3 mg/l BA for 4 weeks.

trimming in longitudinal sections	Average of new shoot (shoot)*
Not trimmed (control)	2.20 c
2 sections	4.30 a
3 sections	3.50 b

* significant difference ($p \leq 0.05$),
a-c Average compared mean within a column by Duncan's multiple range test ($p \leq 0.05$)

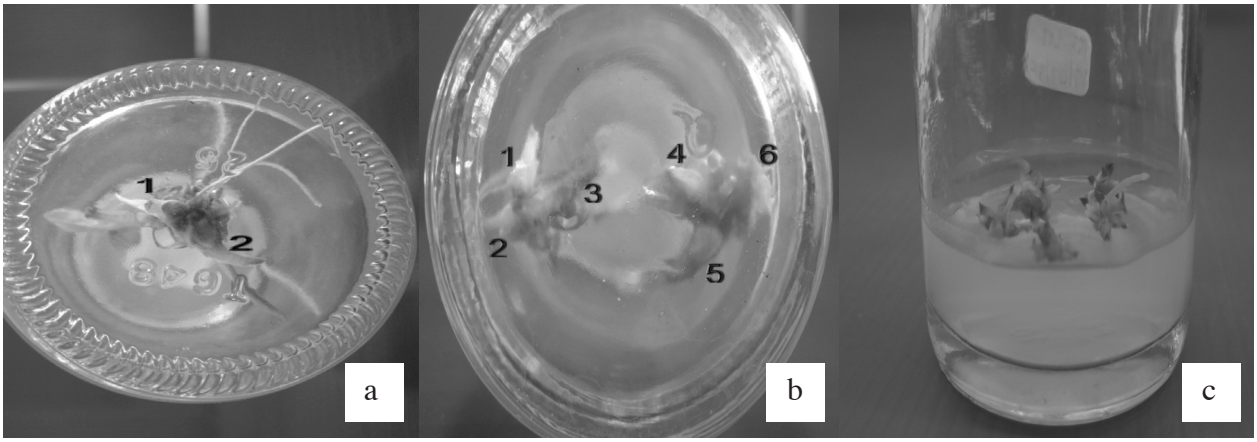

Fig. 1. New shoots regenerated from sections which were cultured on MS medium supplemented with 3 mg/l BA for 4 weeks a) not trimmed b) 2 sections c) 3 sections .

Reference [2] reported a proliferation rate of 3.43 shoot/bud from Curcuma sp. after growing the terminal bud on MS medium supplemented with 13.32 μMBA for 4 weeks. [17] also reported that shoot multiplication rates of 4.2, 3.5, and 6.6 shoots/explant for 8 weeks in liquid medium supplemented with 1 μM NAA and BA, kinetin or 2iP (10μM each). References [10] and [11] also reported a high frequency shoot multiplication (18.22 shoots/explant) after culturing terminal bud explants on MS medium supplemented with 18.17 μM thidiazuron for 12 weeks. [14] reported that trimmed shoot tips of Curcuma sparnifolia Gagnep increased new shoot tips more than with no trimming. References [12] and [13] had trimmed Curcuma paviflora hybrid to 2, 3, and 4 sections. The result showed that 2 sections gave the highest new shoots, and 3 sections was second.

3.3 Effect of sucrose concentration for shoot induction

Cultured young shoot tips of about 1 cm. long on MS medium were supplemented with varied concentrations of (3, 4, 5, 6 and 7 %) for 12 weeks. The results showed that plant grew up and there were significant differences ($p \leq 0.05$) between their parameters, except number of roots was not significantly different (Table 3). However, a significant increase in shoot length, number of leaves, leaf width, and size of roots were observed. MS medium, supplemented with 6% sugar, gave the highest average new shoots (13.4 shoots) per bunch and the number of leaves per bunch, leaf width, and size of roots, were, 56.8 leaves, 2.25 cm, and 2.1 cm, respectively. Number of roots in each treatments were 1.7-2.1. MS medium supplemented with 4% gave the highest average number of root length as 14.15 cm. Sucrose is widely used as a standard carbon source for plant tissue culture, and different concentrations and different osmotic environments have been used. References [1], [3], [17], and [20] reported that MS medium supplemented with 6% sucrose led to increased turmeric plant size, leaves and roots. Reference [4] reported that 2% sugar (a low concentration) is best for in vitro multiplication of Curcuma sp. However, a high concentration of sugar has been found to be ideal for in vitro microrhizome production in Zingiber officinale [23], but [15] produced microrhizomes turmeric on MS medium supplemented with 10% sucrose.

Table 3 Effect of concentration of sugar in MS medium on number of shoots per bunch, number of leaves per bunch, leaf width, leaf petiole length, leaf length, number of roots, root length, and size of roots of Curcuma longa L., after culturing for 12 weeks.

Sucrose Conc.	No. of shoots / bunch*	No. of leaves/ bunch*	Leaf width*	Leaf Petiole Length*	Leaf length*	No. of roots ns	Root length*	Size of roots*
0%	3.2^{f}	12.6^{f}	2.5^{b}	9.7^{ab}	7.6^{a}	0.2	0.51^{f}	1^{b}
3%	6.9^{e}	39.8^{e}	2.71^{a}	10.81^{ab}	8.7^{b}	1.7	1.53^{bc}	1^{b}
4%	8.9^{d}	46.5^{c}	1.89^{b}	10.42^{ab}	9.09^{b}	1.6	14.15^{a}	1.2^{b}
5%	11.9^{b}	44.9^{d}	2.07^{b}	11.0^{a}	8.9^{b}	1.9	11.75^{b}	1.5^{ab}
6%	13.4^{a}	56.8^{a}	2.25^{b}	8.65^{ab}	7.175^{b}	1.8	8.9^{d}	2.1^{a}
7%	10.7^{c}	50.5^{b}	2.2^{b}	7.25^{c}	9.1^{b}	2.1	10.6^{e}	2.1^{a}

* Significant difference ($P \leq 0.05$)

a-f Average compared mean within a column by Duncan's multiple range test at ($p \leq 0.05$)

size of roots : small and thin, diameter about 0.5 - 1 mm = 1, medium, diameter about 1-1.5 mm = 2, Large, diameter more than 1.5 mm = 3,

Number of roots : 1 - 5 roots = 1, 6 -10 roots = 2, More than 11 roots = 3

4. Conclusion

Shoot tips of Curcuma longa L., about 0.5 – 1 cm long, were used as explants. These explants were cultured on MS medium supplemented with varied concentration of BA (1, 2, 3, and 4 mg/l) and NAA (0, 0.5, and 1 mg/l) for the initial study. Clean cultured explants were subcultured every 3 weeks in the same culture for 4 times. Explants grew up and all parameters were significantly different among treatments. The highest average number of new shoots (2.6 and 2.4 new shoots) came from explants which were cultured on MS medium supplemented with 1 mg/l NAA and 2, 3 mg/l BA, (respectively). MS medium supplemented with only 2 mg/l BA produced the highest average number of new shoots (2.6 shoots), number of leaves (5.4 leaves), optimum number of roots (2.6 roots per shoot), and plant height (4.5 cm).

After trimming shoot tips in longitudinal sections to 2 and 3 sections, and culturing on MS medium supplemented with 3 mg/l BA, many new shoots were regenerated at the base of sections. There was a significant difference ($p \leq 0.05$) between their treatments. Shoot tips which were trimmed in half (2 sections) gave the highest number of new shoots (4.3 shoots) when compared to the control (2.2 shoots), and trimmed in 3 sections (3.5 shoots) was second. MS medium supplemented with 6 % sugar was significantly different ($p \leq 0.05$) and gave the highest average on number of shoots, leaves per bunch, leaf width, leaf petiole length, leaf length, number of roots, root length, and size of roots.

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