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# 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 tip of *Curcuma longa* L. were used as explants. These explants were cultured on MS medium supplemented with various concentration 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 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 leaf (5.4 leaves), optimum number of roots ( 2.6 roots per shoot) and plant height (4.5 cm). In 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. When trimmed explants in longitudinal section (LS) to 2 and 3 sections. At 2 sections gave the highest number of new shoots (4.3 shoots per section). Explants which cultured on MS medium with 60 gm/l gave the highest average shoots and leaves per bunch, longest and biggest size of root.



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## 1. Introduction

*Curcuma longa* L. is belong to Family Zingiberaceae , produces edible rhizome which use as a medicine and food additive. Rhizome was used for peptic ulcer (Prucksunand et al,.2001), gastic ulcer (Kositchaiwat et al., 1993) and dyspepsia (Thamlikitkul et al., 1989). Micropropagation gave many plantlets which true to type and provided uniform plants. (George, 1993). High quality of *Curcuma longa* with high and constant level of curcuminoids and volatile oil were wanted to get multiple their plants. *In vitro* propagations of *Curcuma longa* have been done (Yasuda *et al,*. 1988, Salvi *et al.,* 2000, 2001, 2002 and Prathanturarug et al., 2003, 2005).

This work involved investigations about size of shoot tip which trimmed in small pieces and studied the effect of BA, NAA and sucrose concentration which effected on number of new shoots and new roots for large scale propagation.

## 2. Materials and Method

MS medium was used as culturing medium, sprout of young shoots tip 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 (0, 1, 2, 3, 4 mg/l) BA and (0, 0.5, 1 mg/l) NAA for initiation, elongation, regeneration and shoot bud formation. The second experiment: Shoot tip about 0.5 cm long were used for trimming in longitudinal section with half (2 sections) and 3 sections per shoot tip. The third experiment: Shoot tip 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±2ºC under cool white florescent light ($37\mu molm^{-2}\ S^{-1}$) for 16/8 h. photoperiod.

## 3. 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 with 25 replications per treatments.

## 4. Result and Discussion

After cultured explants on MS medium with vary concentration of (1, 2, 3 and 4 mg/l) BA and (0, 0.5 and 1 mg/l) NAA for initial studied. Clean cultured 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 as:

### 4.1 Effect of NAA and BA on growth of plantlet

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

**Table 1:** Average number of new shoot, root, leaf and plant height of *Curcuma longa* L. on MS medium supplemented with combination of BA and NAA after cultured for 12 weeks.

| MS supplemented with | | number of new shoot* | number of leaf* | number of root* | 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 b c- Average compared mean within column by Duncan's multiple range test ($p \leq 0.05$)

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

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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 Shukla *et al*.(2007) had done with *Curcuma angustifolia* Rozbi which used 3 mg/l BAP could produced 6.9 shoots per explants within 6 weeks . Nasirujjaman *et al*.(2005) had done with *Curcuma longa* and cultured young shoot on WPM medium supplemented with 4 mg/l BAP which was the best medium to regenerated new shoot (6.25 shoots per plant) within 2 weeks. In *Zingiber officinale* Rosc. which related family to turmeric, was reported by Balachandran *et al* (1990). Malamug *et al.* (1991) and Sunitibala *et al*. (2001) 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.

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

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

* Significant difference (P≤ 0.05)

abc - Average compared mean within column by Duncan's multiple range test at (p≤ 0.05).

## 4.2 Induced new shoots from trimming shoot tip

After trimming shoot tip in longitudinal section to 2 and 3 sections, all of sections were cultured on MS medium supplemented with 3 mg/l BA. After cultured the section of shoot tip for 4 weeks, many new shoots were regenerated at the base of section which contract to the medium in each sections and the result were recorded in Table 2. When compared the average number of new shoots which regenerated from each sections. It was significant difference ( p≤ 0.05) from each other. Shoot tip which 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.

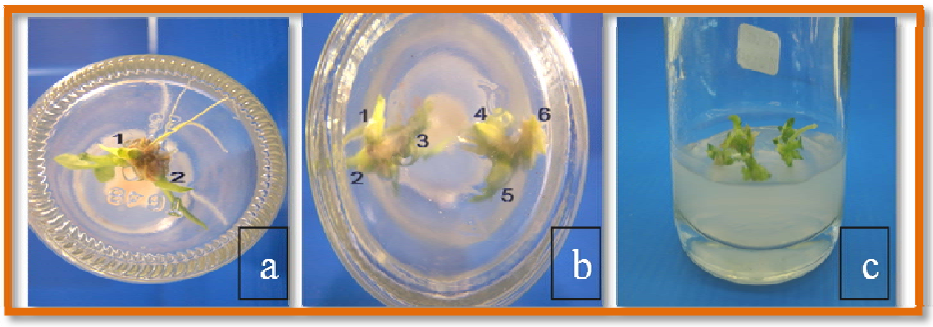


**Figure 1:** New shoots regenerated from section which cultured on MS medium supplemented with 3 mg/l BA for 4 weeks a) no trimmed b) 2 section c) 3 sections.

As Balachandran *et al.* (1990) 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. Salvi *et al.* (2002) also reported that shoot multiplication rates of 4.2, 3.5 and 6.6 shoots/explants for 8 weeks in liquid medium supplemented with 1µM NAA and BA, kinetin or 2iP (10µM each), respectively. Prathanturarug et al. (2003, 2005) also reported a high frequency shoot multiplication (18.22 shoots/explants) after culturing terminal bud explants on MS medium supplemented with 18.17 µM thidiazuron for 12 weeks. Ora-Ubon (1991) reported that trimmed shoot tip of *Curcuma sparnifolia* Gagnep were increased new shoot tip more than no trimming. Phaephun et al. (2006, 2007) had trimmed *Curcuma paviflora* hybrid to 2, 3 and 4 section. The result showed that 2 sections gave the highest new shoots and 3 sections was the second.

## 4.3 Effect of sucrose concentration for shoot induction

When cultured young shoot tip about 1 cm. long on MS medium supplemented with vary concentration of (3, 4, 5, 6 and 7%) sugar for 12 weeks. The result showed that plant grew up and their were significant different ($p \leq 0.05$) between their parameter except number of root was not significant difference (Table 3). However, a significant difference increases in shoot length, number of leaf, leaf width and size of root were observed. In MS medium supplemented with 6% sugar gave the highest average new shoots per bunch also number of leaves per bunch, leaf width and size of root where as 13.4 shoots, 56.8 leaves, 2.25cm, and 2.1cm, respectively. Numbers of roots in each treatment were 1.7 – 2.1. In MS medium supplemented with 4 % gave the highest average number of root length where 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. Barthakur and Bordoloi

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(1992), Sanghamitra and Nayak (2000) Sunitibala *et al.* (2001) and Adelberg and Cousins(2007) reported that MS medium supplemented with 6% sucrose led to increased turmeric plant size, leaf and root. (As Das *et al*. (2010) reported that 2% sugar that low concentration of sugar could be best for *in vitro* multiplication of *Curcuma* sp. However high concentration of sugar source has been found to be ideal for *in vitro* microrhizome production in *Zingiber officinale* ( Zheng *et al*., 2008), but Raghu (1997) produced microrhizomes turmeric on MS medium supplemented with 10%sucrose.

**Table 3:** Effect of concentration of sugar in MS medium on number of shoot per bunch, number of leaf per bunch, leaf width, leaf petiole length, leaf length, number of root, root length, and size of root of *Curcuma longa* L. after cultured for 12 weeks.

| Sucrose Conc | No. of shoot/bunch * | No. of leaves/bunch* | Leaf width* | Leaf Petiole Length* | Leaf length* | No. of root ns | Root length* | Size of root* |
|---|---|---|---|---|---|---|---|---|
| 0 | 3.2f ±0.971 | 12.6f ±4.540 | 2.5b ±00.098 | 9.7ab ±0.405 | 7.6 a ±0.366 | 0.2 ±0.152 | 0.51f ±1.943 | 1b ±0.006 |
| 3% | 6.9e ±2.377 | 39.8e ±9.070 | 2.71a ±0.228 | 10.81ab ±0.977 | 8.7 b ±0.777 | 1.7 ±0.163 | 1.53 e ±1.955 | 1 b ±0.133 |
| 4% | 8.9d ±2.280 | 46.5c ±4.230 | 1.89b ±0.102 | 10.42ab ± 0.453 | 9.09b ±0.679 | 1.6 ±0.314 | 14.15 a ±1.824 | 1.2 b ±0.166 |
| 5% | 11.9b ±3.528 | 44.9d ±11.27 | 2.07b ±0.351 | 11.0 a ±1.115 | 8.9b ±0.619 | 1.9 ±0.20 | 11.75b ±1.608 | 1.5ab ±0.276 |
| 6% | 13.4a ±1.653 | 56.8a ±6.130 | 2.25b ±1.097 | 8.65 ab ±0.922 | 7.175a ±0.28 | 1.8 ±0.29 | 8.9d ±1.654 | 2.1 a ±0.314 |
| 7% | 10.7c ±1.046 | 50.5b ±3.339 | 2.2b ±0.453 | 7.25c ±0.047 | 9.1b ±0.471 | 2.1 ±1.044 | 10.6c ±0.843 | 2.1a ±0.110 |

* Significant difference (P≤ 0.05)

abc - Average compared mean within column by Duncan's multiple range test at (p≤ 0.05)

Size of root: Small and thin, diameter about 0.5- 1mm = 1,
Medium: diameter about 1-1.5 mm = 2,
Large: diameter more than 1.5 mm = 3,

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

## 5. Conclusion

Shoots tip of *Curcuma longa* L. about 0.5–1cm long were used as explants. These explants were cultured on MS medium supplemented with vary concentration of (1, 2, 3 and 4 mg/l) BA and (0 0.5 and 1 mg/l) NAA for initial studied. Clean cultured of explants were subcultured every 3 weeks in the same culture for 4 times. Explants grew up and all parameters were significant difference among treatments. The highest average number of new shoots (2.6 and

2.4 new shoot) were came from explants which cultured on MS medium supplemented with 1mg/l NAA and 2, 3mg/l BA, (respectively) and MS medium supplemented with only 2mg/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).

After trimming shoot tip in longitudinal section to 2 and 3 sections, and cultured on MS medium supplemented with 3 mg/l BA. Many new shoots were regenerated at the base of sections. It was significant different ($p \leq 0.05$) between their treatments. Shoot tip which 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. MS medium supplemented with 6 % sugar was significant different ($p \leq 0.05$) and gave the highest average on number of shoot and leaf per bunch, leaf width, leaf petiole length, leaf length, number of root, root length, and size of root**.**

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