
Micropropagation of young inflorescence *Curcuma* hybrid *In vitro*

Yoosumran, V.¹, Saetiew, K.^{1*}, Ruamrungsri, S.², Akarapisarn, A.³ and Teerarak, M.¹

¹Department of Plant Production and Technology, Faculty of Agricultural Technology King Mongkut's Institute of Technology Ladkrabang, Chalongkrung Rd., Bangkok 10520; ²Department of Plant and Soil Science, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand; ³Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand.

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Abstract Five cultivars of *Curcuma* hybrid consisted of 3 *Paracurcuma* and 2 *Eucurcuma* young inflorescences were cultured on MS medium supplemented with 0, 0.5 and 1 mg/l NAA and BA for 8 weeks. It was found that young inflorescences *Paracurcuma* hybrid *Curcuma* 'Yuki' cultured on MS medium supplement with the 1 mg/l NAA and 0.5 mg/l BA were able to induce the most shoots at 2.67 shoots, with the highest percentage of shoots (80.00%) and the average plant height is 5 cm. For *Curcuma* 'Burgundy' and *Curcuma* 'Phonphisit' cultured on MS medium supplement with 1 mg/l NAA and 1 mg/l BA, they were able to induce the most shoots at 1.66 and 1.78 shoots, the highest percentage of shoots is 73.33 and 76.67% respectively and the average plant height is 5 cm. The other cultivars *Eucurcuma* consist of *Curcuma* 'Sweetmemory' and *Curcuma* 'Banrai Red'. The young inflorescences were divided into 2 parts, the upper and the lower one. The result shows that the lower shoot induction is better than upper one. *Curcuma* 'Sweetmemory' grown on MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA were able to induce the most shoots at 2.38 shoots, and the highest percentage of shoots is 83.33%. *Curcuma* 'Banrai Red' grown on MS medium supplemented with 0.5 mg/l NAA and 1 mg/l BA were able to induce the most shoots at 1.98 shoots, the highest percentage of shoots is 63.33% and both cultivars were the same height average at 5 cm.

Keywords: *Paracurcuma*, *Eucurcuma* and micropropagation

Introduction

Pathumma and Krachiew were planted in the same family as ginger and galangal (*Zingiberaceae*) in the genus *Turmeric* (*Curcuma* sp.) originated in Indochina, Myanmar and Thailand. In Thailand, Pathumma can be seen in almost every region of the country but mostly found in the northern region and northeast which have the most variety of species. Botanists have classified this

* **Corresponding Author:** Saetiew, K.; **Email:** kanjana.sa@kmitl.ac.th

genus into 2 sub-genus, namely the subgenus *Paracurcuma* or the Pathumma group and *Eucurcumar* or Krachiew group (Bunya-atichart *et al.*, 2004). The distinctive features of the Pathumma group are white or purple true mouth petals and the inflorescence flowering is caused by artificial stems long peduncle bud shoots with a number of basic chromosomes in the range of 12-18 sticks. The sub-genus *Eucurcumar* or Krachiew group distinctive features are white or yellow true mouth petals and the inflorescence flowering is caused by the rhizome directly, inflorescences from the tops of artificial stems short peduncle inflorescences are large and has 21 sticks of basic chromosomes.

These ornamental plants become more popular not only in Thailand but also in foreign countries. The annual exported value of rhizomes is about 20-30 million baths (Salakpetch, 2020). The major markets are the United States of America, Japan, the Netherlands, Italy, China and Belgium (Someyo and Authlungrong, 2020). At present, the plant tissue culture technology has greatly played a role in facilitating shoot production due to the fact that the tissue culture can help in large amount of shooting in a short time and free from disease depending on the plant growth regulators. There are several factors effecting the proliferation of *Paracurcuma* and *Eucurcuma* in sterile conditions. One of them that effects growth and tissue change of the shoot or the root is the balance of the auxin and cytokinin hormones. There are several research papers investigating the effects of hormones on growth and development in Ginger plants (*Zingiberaceae*) such as Prathanturarug *et al.* (2005) who studied on increasing the number of turmeric shoots by cultured the bud explants for 1 week in liquid MS medium supplemented with TDZ concentrations 72.64 μM before cultured in solid MS. After that, the growth regulators were added for 8 weeks. The result showed that the rate of new plant growth was increased to 11.4 shoots/explant. Ferdous *et al.* (2012) studied on the propagation of wild white turmeric from bud and rhizomes *In vitro*. They were cultured in MS medium enriched with BA or Kinetin at concentrations 0, 2, 4, 6, 8 and 10 μm alone or in supplement with NAA and IBA at concentrations 1, 1.5 and 2 μm . It was found that when cultured in MS medium with 8 μm BA and 1 μm NAA induced maximum shoots of 10 shoots/explant. Therefore, the project investigated the young inflorescence of *Paracurcuma* and *Eucurcuma* and studied the effect of NAA and BA concentration which affected the number and the percentage of the new shoots.

Materials and methods

Preparations of MS medium

Murashige and Skoog (1962) (MS) medium is supplemented with 3% sucrose, 8 g/l agar, 0, 0.5 and 1 mg/l NAA and 0, 0.5 and 1 mg/l BA. The pH was adjusted to 5.5-5.8 prior to autoclaving at 121 °C for 20 min.

Preparations of initial explants

The total 5 young inflorescence cultivars of three *Paracurcuma* consisted of Yuki, Bergundy and Pornpisith, and two *Eucurcuma* consist of Sweetmemory and Banrai Red. The young inflorescences were washed thoroughly under running water for 30 min. After that they were surface sterilized with 20% clorox (1.2% Sodium hypochloride) tween-20 added 1-2 drop for 20 min, sterilized with 5% clorox (0.3% sodium hypochloride) tween-20 added 1-2 drop for 5 min and being washed 3 times with sterilize distilled water for 5 min.

Shoot induction

The young inflorescence of *Paracurcuma* explants (size 0.5-1 cm) were cultured on the modified Murashige and Skoog (1962) (MS) medium supplemented with 0, 0.5 and 1 mg/l NAA and 0, 0.5 and 1 mg/l BA to induced shoot. The explants were cultured under cool fluorescent lamps at light intensity of 40 $\mu\text{Molm}^{-2}/\text{sec}$ with a 16 hours light at temperature 25 ± 2 °C. The experiment was performed in completely randomized design (CRD) with 9 treatments. Each treatment consisted of 10 explants in *Paracurcuma* (Yuki, Bergundy and Pornpisith).

The young inflorescence of *Eucurcuma* was separated into 2 parts, the upper and the lower inflorescence. They were also cultured on the MS medium with same concentration of NAA and BA. The experiment was 9×2 factorial in randomized complete design, 18 treatments, 3 replications and 10 explants per replication in *eucurcuma* (Sweetmemory and Banrai Red).

The shoots were transferred to the fresh medium after 30 days. The effectiveness of plant growth regulators was evaluated from the number of new shoots, the percentage of shoot regeneration and the shoot length.

Statistical analysis

All data were analyzed by using ANOVA and Duncan's multiple range tests at $p \leq 0.05$ by SAS programme.

Results

The effect of NAA and BA on the growth of the young inflorescence Paracurcuma (Yuki, Bergundy and Pornpisith)

The inflorescences of all 3 *Paracurcuma* cultivars consisted of Yuki (Figure 1A), Bergundy (Figure 1B) and Pornpisith (Figure 1C). After culturing

them for 3 weeks, it was found that the young inflorescences were grown with larger initial parts and the color of explant changed to green color. The new shoots were also evolved. After cultured 8 weeks of Yuki it was found the effects of various plant growth regulators on percentage of shoot regeneration, shoot number and shoot length are showed in Table 1. The Yuki explants which were cultured on MS medium supplemented with 1 mg/l NAA and 0.5 mg/l BA was the most effective for induce the most shoot 2.67 shoots per piece, had the highest percentage of shoot 80% and average shoot length 5 cm (Table 1, Figure 2B). The young inflorescences of Bergundy and Pornpisith on MS medium supplemented with 1 mg/l NAA and 1 mg/l BA can induce the most shoot at 1.66 shoots and 1.78 shoots respectively, had the highest percentage of shoots 73.33% and 76.67% respectively and 2 cultivars had average shoot length 5 cm (Table 2 and 3, Figure 2D and 2F). In addition, all 3 cultivars, when compared with the non treated growth regulators which were able to regenerate shoot at 46.67%. The new sprouts were likely less than those treated with growth regulators (Figure 2A, 2C and 2E).

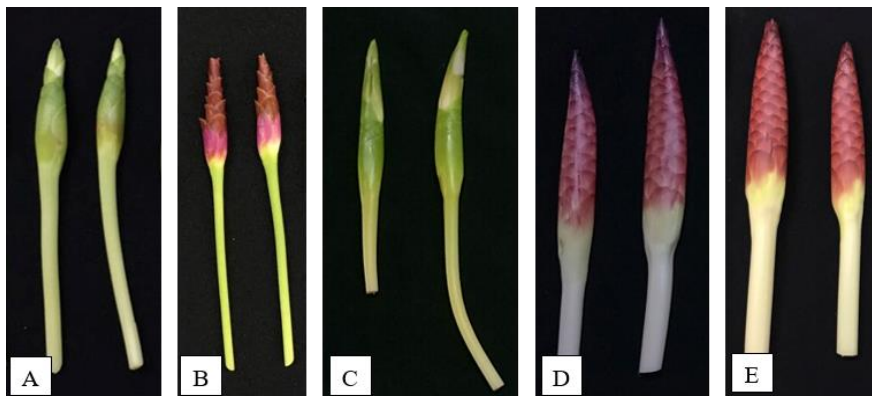


Figure 1. Five cultivars of curcuma hybrid: 3 paracurcuma young inflorescences (A) Yuki, (B) Bergundy, (C) Pornpisith, 2 eucurcuma (D) Sweetmemory and (E) Banrai Red

The effect of NAA and BA on the growth of the young inflorescence Eucurcuma (Sweetmemory and Banrai Red)

The inflorescences of 2 Eucurcuma cultivars consisted of Sweetmemory (Figure 1D) and Banrai Red (Figure 1E). After being sterilized, the young inflorescences are divided into 2 parts, the upper (Figure 3A and 4A) and the lower one (Figure 3C and 4C). The piece of inflorescences were cut to 0.5-1 cm. After 3 weeks culturing, it was found that the lower inflorescence was induced the shoot better than upper one. The young inflorescence of

Sweetmemory cultured on MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA can induce the most shoots at 2.38 shoots, with the highest percentage of shoot 83.33% and the average plant length was 5 cm (Table 4, Figure 3E and 3F). The young inflorescences of Banrai Red on MS medium supplemented with 0.5 mg/l NAA and 1 mg/l BA can induce the most shoots at 1.98 shoots, with the highest percentage of shoots 63.33% and the average plant length was 5 cm (Table 4, Figure 4E and 4F). In addition, when compared the explants which were cultured on MS free plant growth regulators, the result showed that the 2 *Eucurcuma* cultivars can induced shoots similarly. However, the new sprouts from culturing in free growth regulator were less than those treated with growth regulators (Figure 3B, 3D, 4B and 4D).

Table 1. The effect of NAA and BA on the growth of the young inflorescences *paracurcuma* hybrid *curcuma* ‘Yuki’ after 8 weeks

plant growth regulator (mg/l)		% Shoot regeneration	Shoot No.	Shoot length (cm)
NAA	BA			
0	0	46.67±5.77c	1.11±0.19d	5.05±0.05
0	0.5	53.33±5.77bc	1.33±0.19bcd	5.05±0.05
0	1	46.67±5.77c	1.22±0.19cd	5.05±0.04
0.5	0	53.33±5.77bc	1.33±0.19bcd	5.04±0.05
0.5	0.5	60.00±0.00b	1.55±0.19ab	5.06±0.05
0.5	1	56.67±5.77bc	1.44±0.19bc	5.06±0.05
1	0	53.33±5.77bc	1.33±0.19bcd	5.03±0.05
1	0.5	80.00±0.00a	2.67±0.57a	5.04±0.05
1	1	56.67±5.77bc	1.44±0.19bc	5.03±0.05
F-test		**	**	ns
CV.		9.73	11.33	1.02

ns = non significant difference ** Significant different at $P \leq 0.01$

Means within column followed by the same later are not significant different as determined by Duncan's multiple range test

Table 2. The effect of NAA and BA on the growth of the young inflorescences *Paracurcuma* hybrid curcuma ‘Bergundy’ after 8 weeks

plant growth regulator (mg/l)		% Shoot regeneration	Shoot No.	Shoot length (cm)
NAA	BA			
0	0	53.33±5.77b	1.11±0.38b	5.01±0.01
0	0.5	56.67±5.77b	1.33±0.00ab	5.02±0.01
0	1	63.33±5.77ab	1.44±0.19ab	5.01±0.01
0.5	0	56.66±5.77b	1.11±0.38b	5.02±0.04
0.5	0.5	63.33±5.77ab	1.44±0.19ab	5.00±0.01
0.5	1	63.33±5.77ab	1.33±0.00ab	5.06±0.04
1	0	63.33±5.77ab	1.33±0.00ab	5.02±0.04
1	0.5	56.67±5.77b	1.44±0.19ab	5.03±0.03
1	1	73.33±5.77a	1.66±0.33a	5.02±0.04
F-test		*	*	ns
CV.		9.44	17.69	0.61

ns = non significant difference * Significant different at $P \leq 0.05$

Means within column followed by the same later are not significant different as determined by Duncan’s multiple range test

Table 3. The effect of NAA and BA on the growth of the young inflorescences *Paracurcuma* hybrid curcuma ‘Pornpisith’ after 8 weeks

plant growth regulator (mg/l)		% Shoot regeneration	Shoot No.	Shoot length (cm)
NAA	BA			
0	0	50.00±0.00c	0.78±0.19c	5.07±0.04
0	0.5	53.33±5.77c	1.11±0.1bc	5.06±0.04
0	1	50.00±0.00c	0.89±0.19bc	5.06±0.04
0.5	0	53.33±5.77c	1.11±0.19bc	5.08±0.01
0.5	0.5	63.33±5.77b	1.11±0.19bc	5.08±0.01
0.5	1	63.33±5.77b	1.11±0.19bc	5.08±0.01
1	0	53.33±5.77c	1.11±0.19bc	5.09±0.00
1	0.5	63.33±5.77b	1.22±0.19b	5.08±0.01
1	1	76.67±5.77a	1.78±0.19a	5.05±0.05
F-test		**	**	ns
CV.		8.70	16.77	0.64

ns = non significant difference ** Significant different at $P \leq 0.01$

Means within column followed by the same later are not significant different as determined by Duncan’s multiple range test

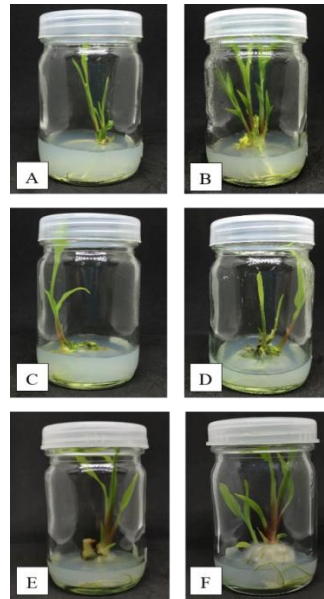


Figure 2. Paracurcuma was cultured after 8 weeks (A) Yuki control, (B) Yuki MS+1 mg/l NAA+0.5 mg/l BA, (C) Bergundy control, (D) Bergundy MS+1 mg/l NAA+1 mg/l BA, (E) Pornpisith control and (F) Pornpisith MS+ 1 mg/l NAA+1 mg/l BA

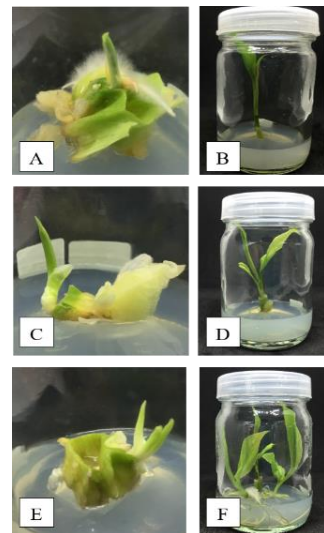


Figure 3. The effect of young inflorescences part and plant growth regulator NAA and BA in Sweetmemory in 3 and 8 weeks (A and B) the upper part of young inflorescences and free growth regulator, (C and D) the lower part and free growth regulator (E and F) the lower part and 0.5 mg/l NAA+ 0.5 mg/l BA

Table 4. Shooting response in young inflorescence part of flower of *Eucurcuma* (Curcuma ‘Sweetmemory’ and ‘Banrai Red’) and the treatment of NAA and BA

Plant growth regulator (mg/l)		Sweetmemory				Banrai Red			
NA	B	Part ¹	%Shoot regeneration	Shoot No.	Shoot length (cm)	%Shoot regeneration	Shoot No.	Shoot length (cm)	
A	A	/							
0	0	a	40.00±0.00e	0.89±0.19d	5.05±0.0	36.66±1.54	0.78±0.19c	5.08±0.0	
		b	40.00±0.00e	0.89±0.19d	5.00±0.0	36.66±5.77c	0.78±0.19c	5.08±0.0	
0	0.5	a	40.00±0.00e	0.89±0.19d	5.06±0.0	40.00±0.00d	0.89±0.19c	5.09±0.0	
		b	40.00±0.00e	0.89±0.19d	5.06±0.0	53.33±5.77b	1.11±0.19b	5.08±0.0	
0	1	a	40.00±0.00e	0.89±0.19d	5.05±0.0	40.00±0.00d	0.89±0.19c	5.08±0.0	
		b	40.00±0.00e	0.89±0.19d	5.04±0.0	53.33±5.77b	1.11±0.19b	5.06±0.0	
0.5	0	a	43.33±5.77de	1.00±0.00c	5.06±0.0	46.66±5.77c	1.00±0.00c	5.09±0.0	
		b	43.33±5.77de	1.00±0.00c	5.06±0.0	46.66±5.77c	1.00±0.00c	5.09±0.0	
0.5	0.5	a	53.33±5.77bc	1.33±0.33b	5.04±0.0	53.33±5.77b	1.11±0.19b	5.08±0.0	
		b	83.33±5.77a	2.38±0.38a	5.04±0.0	53.33±5.77b	1.11±0.38b	5.07±0.0	
0.5	1	a	50.00±0.00bc	1.22±0.19c	5.03±0.0	43.33±5.77d	1.11±0.38b	5.06±0.0	
		b	56.67±5.77b	1.55±0.19b	5.03±0.0	63.33±5.77a	1.98±0.19a	5.06±0.0	
1	0	a	43.33±5.77de	1.00±0.00c	5.05±0.0	46.66±5.77c	1.00±0.00c	5.08±0.0	
		b	46.66±5.77cd	1.11±0.19c	5.03±0.0	56.67±5.77a	1.44±0.19a	5.08±0.0	
1	0.5	a	43.33±5.77de	1.00±0.00c	5.06±0.0	40.00±0.00d	0.89±0.19c	5.08±0.0	
		b	50.00±0.00bc	1.22±0.19c	5.00±0.0	46.66±5.77c	1.00±0.19c	5.09±0.0	
1	1	a	40.00±0.00e	1.00±0.00c	5.04±0.0	40.00±0.00d	0.89±0.19c	5.08±0.0	
		b	43.33±5.77de	1.11±0.19c	5.05±0.0	53.33±5.77b	1.11±0.19b	5.06±0.0	
F-test A			**	**	ns	**	**	ns	
F-test B			*	**	ns	**	**	ns	
F-test A*B			**	**	ns	**	**	ns	
CV%			8.78	16.40	0.98	10.78	19.30	0.53	

ns = non significant difference * Significant different at P≤ 0.05 ** Significant different at P≤0.01
Means within column followed by the same later are not significant different as determined by Duncan's multiple range test
¹a= upper part of young inflorescences, b= lower part of young inflorescences

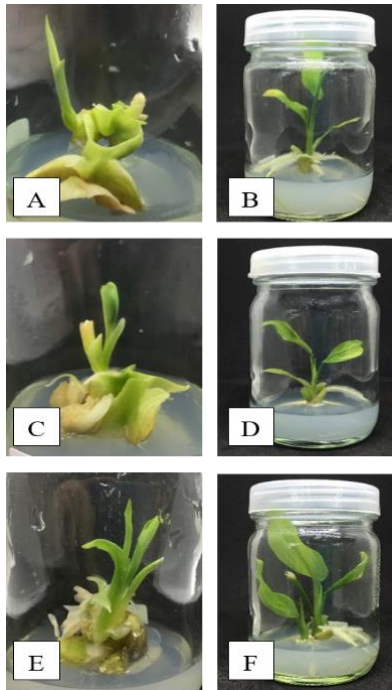


Figure 4. The effect of young inflorescences part and plant growth regulator NAA and BA in Banrai Red in 3 and 8 weeks (A and B) the upper part of young inflorescences and free growth regulator, (C and D) the lower part and free growth regulator (E and F) the lower part and 0.5 mg/l NAA+ 1 mg/l BA

Discussion

This study was investigated the 3 varieties of *Paracurcuma* young inflorescence and 2 varieties of *Eucurcuma* micropropagation on the effect of NAA and BA. The percentage of shoot regeneration was examined in young inflorescence of *Paracurcuma* cultured on MS medium containing NAA and BA in 8 weeks. The findings indicated that the shoot induction and the number of shoots can be found in the medium containing combination of NAA and BA than the free one or the one with only one plant growth regulator. NAA is a hormone in the group of auxin. Auxin has a stimulating effect on cell enlargement, cell elongation and the root formation (Jafrai *et al.*, 2016). NAA has a relatively high auxin activity and is highly mobile but decay slowly, thus stimulating the good root (Adriance and Brison, 1955). BA is a hormone in the group of cytokinin. Cytokinin effect the division cells which help the growth of the plant, stimulate cell division by stimulating the synthesis of essential proteins in the translation phase, it promotes ribosome formation in the cell cycle, which influences crest induction and stimulate the growth of the lateral

bud (Brock and Kaufman, 1991). The result also revealed that Paracurcuma and Eucurcuma can induce shoots best when NAA and BA are used together. This is because the proportion of plant growth regulators in both groups is an important determinant. The high ratio of cytokinin to auxin stimulates the shoot formation. But if the ratio of cytokinins to auxins is low, the tissue develops into roots. However, in some plants, the addition of growth regulators to a single cytokinin group can lead to tissue development for both shoots and roots. As a result, the plant hormones or hormones accumulate in the tissue can act as a stimulant and participate in various processes leading to normal development. Therefore, the addition of growth regulators to the diet may not always be necessary (Skoog and Miller, 1957).

In this study the root induction can be found in all shoots. The presence of both plant and root occurs in the same diet due to NAA, which is classified as a growth regulator in the auxin group. It plays a role in regulating cell enlargement stimulates cell division and the root growth (Kakani *et al.* 2009). Plant tissue culture use to be the technique for propagation. The cytokinin such as BA are commonly used to induce plantlets. (Kaviani *et al.* 2015). Nasirujjaman *et al.* (2005) studied the micropropagation of Turmeric (*Curcuma longa* Linn.) through *In vitro* rhizome bud culture. It was showed that the MS medium supplemented with 1 mg/l NAA and 4 mg/l BAP gave the highest average new shoots. The effect of young inflorescences part explant and the concentration of growth regulators was determined in Eucurcuma 2 varieties. The lower of inflorescence response shoot regeneration than upper part. The type and dosage of the growth regulator given to the medium is very important to be considered in order to induce the development of explants in the desired direction (Zulkarnain *et al.*, 2019). In this experiment, the application of combination 0.5 mg/l NAA and 0.5 mg/l BA was able to increase the highest percentage of shoot regeneration and the shootnumber of Sweetmemory variety. This combination also gave the best result for Banrai Red variety. The same result has shown in research of Bharalee *et al.* (2005) who also found that 4.0 mg/l BA + 1.5 mg/l NAA were the best growth regulator combination for shoot multiplication of *Curcuma caesia*. A study of Jala (2012), the effects of NAA, BA and sucrose on shoot induction and rapid micropropagation by trimming shoot of *Curcuma longa* L., indicated that the highest new shoots were cultured on MS medium supplemented with 1 mg/l NAA and 2 or 3 mg/l BA. Jala (2014), studied the effect of paclobutrazol and BA on micropropagation in *Curcuma* sp. *In vitro*, showed that both ½ MS and MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA gave the highest average new shoots. In addition, Mujib *et al.* (2008) reported that the addition of BA in combination with NAA effects the growth of new *Caladium Bicolor*

includes the process of embryo formation from somatic cells as well. As well as Ahmed *et al.* (2004) reported that the addition of BA growth regulators with NAA was able to induce a large number of new shoots of *Caladium*.

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