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Studies on micropropagation of *Curcuma caesia* Roxb. (Kunyit Hitam)

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Abstract

Curcuma caesia is a medicinal plant which originated from India. This species contained flavonoids, alkaloids, amino acids, protein, curcuminoids, volatile oils and phenolics. In this study, shoot multiplications were studied. This study is to determine the best plant growth regulators for shoot multiplication. Different concentrations of plant growth regulators such as BAP, KIN and TDZ were studied. The best treatment for shoot multiplication experiment was reported in half strength of MS medium supplemented with 8.0 mg/L BAP with mean of 4.00±0.82 shoots. However, for economic feasibility purposes, 1.0 mg/L with similar result was chosen as the best concentration for shoot induction.

Keywords: *Plant tissue culture; shoot induction, BAP, KIN, TDZ*

1. Introduction

Curcuma caesia Roxb. is originated from India (Velayudhan *et al*., 1999) and native to Bengal. It is a perennial and rhizomatous herb plant which can be differentiated from other Curcuma species through it bluish-black rhizome. It has a bitter and pungent smell (Paliwal *et al*., 2011).

This Curcuma is used as condiments, medicine and perfumes (Kumar, 1991); stimulants, anti-diarrheal, diuretic, anti-emetic, wound cleaner and skin disorder (National Medicinal Plants Board, 2008); fuel source, treat blood diseases, animals and insects bites, congestions and scabies (Velayudhan *et al*. 1999). *C. caesia* was reported to contain more phenolic compound than *C. amada* (Katalinic *et al.*, 2006). The rhizomes of *C. caesia* are consisted of curcuminoids, flavonoids, volatile oil, protein, amino acids and alkaloids (Sarangthem & Haokip, 2010).

Micropropagation is a technique that has many advantages over conventional propagate methods (Prathanturarug *et al.*, 2005). This technique enables the plants to be produced in small space and short time which could help to ensure the availability of Zingiberaceae species for the whole year as it can produce a huge number of plantlets by using a few plants (Hamirah, 2009).

Pranthanturarug *et al.* (2003) revealed that this technique could produce true-to type multiplication of an elite clone in short time. There were many shoot multiplication research which economically reported in Zingiberaceae species such as *C. aromatica*, *C. amada* and *Amomum subulatum* (Prakash *et al*., 2004); *C. domestica* and *C. zedoria* (Yasuda *et al*., 1988; Prakash *et al*., 2004); *C. aeruginosa* (Balachandran *et al*., 1990); *Kaempferia galangal* (Chirangini *et al*., 2005) and ginger (Hosoki and Sagawa, 1977).

The *in vitro* propagation of certain species was affected by type and concentration of plant growth regulator because they played an important role in cell division, differentiation and morphogenesis (Toteva *et al*., 2000). Plant tissue culture technique has offered an alternative pathway in mass production of superior clones in limited time and space which can help in overcome all the problems faced. Therefore, the objective of this study was to optimize the protocol of shoots development using different concentrations and types of plant growth regulators.

2 Materials and methods

2.1 Plant materials

The source of the *in vitro* plants was obtained from the mother plants which planted outside of Plant Tissue Culture Laboratory. Three months old *in vitro* plants that had been maintained in Plant Tissue Culture Laboratory of University Malaysia Sarawak (UNIMAS) were used as explants in all the sub-experiments.

2.2 Shoot multiplications

The *in vitro* plant leaves and roots were dissected. Then, the leafless and rootless shoots were cultured in half strength of MS medium supplemented with different types and concentrations of plant growth regulators. A total of five concentrations of 6- Benzylaminopurine (BAP) which were 1.0, 3.0, 5.0, 8.0 and 10.0 mg/L (Sigma Aldrich, Inc.) were used in this experiment for shoots production. Different concentrations of (Kinetin) KIN and Thidiazuron (TDZ) are also been investigated.

The cultures were kept under white fluorescent tubes providing irradiance of 50µmol m^{-2} s^{-1} for 24 hours photoperiod. The temperature in the culture room was maintained around 25±2°C. The number of shoots, number of leaves, height of shoots (cm) and number of roots were recorded after cultured for three months.

Data collected were analyzed using one-way analysis of variance (ANOVA) followed by mean comparison carried out using Tukey test at $p< 0.05$ levels with SPSS Statistics Version 20.

3. Results

3.1 Effect of BAP on shoots multiplication

The shoot multiplication by using different concentrations of BAP did not show any significant difference between each treatment. The mean number of shoots recorded was varied from 1.30±0.15 to 4.00±0.82. The lowest mean number of shoots was resulted in control treatment while the highest mean number of shoots was recorded in medium supplemented with 8.0 mg/L of BAP (Table 1).

The highest mean number of leaves was produced in medium supplemented with 1.0 mg/L of BAP. The mean recorded by 1.0 mg/L of BAP was higher than control treatment which had mean of 7.80±1.88 (Table 1). The lowest mean number of leaves was obtained in BAP concentration of 10.0 mg/L.

The mean number of shoots height in this experiment was generally decreased from control treatment to 10.0 mg/L of BAP. Although, there was fluctuation for mean number of shoots height in between the treatment. The highest shoot was observed in a control treatment with mean of 10.78±0.60 cm. The shortest shoot was recorded in medium supplemented with 10.0 mg/L of BAP with mean number of 9.49±0.84cm.

The mean number of roots in this experiment was ranged from 4.90±0.50 to 12.05±2.22. The highest mean number of roots was obtained in medium supplemented with 1.0 mg/L of BAP. The medium supplemented with 1.0 mg/L and 8.0 mg/L of BAP had produced brownish green in colour roots whereas 3.0 mg/L of BAP had brownish white colour roots.

3.2 Effect of KIN on shoots multiplication

In this sub-experiment, different concentrations of KIN showed no significant effect for the production of shoots. The concentrations of 3.0 mg/L of KIN had recorded the highest mean number of shoot which was 2.34±0.52 as compared to control treatment (1.30±0.15).While, the KIN concentrations at 1.0 mg/L and 2.0 mg/L had reported the lowest mean number of shoots which was 1.20±0.32 and 1.20±0.36, respectively.

Generally, the mean number of leaves produced in this experiment was increased from control treatment (0 mg/L of KIN) to 4.0 mg/L of KIN. The shoots height in this experiment showed fluctuations trend from control treatment (0 mg/L of KIN) to 4.0 mg/L of KIN. The mean number of roots recorded in this experiment was varied from 4.90±0.50 to 14.75±1.87. The control treatment had showed the lowest significant effect as compared to other treatments except for 0.5 mg/L of KIN treatment. The highest mean number of roots was recorded in 3.0 mg/L of KIN.

3.3 Effect of TDZ on shoots multiplication

Generally, the mean number of shoots was increased from control treatment to 0.4 mg/L of TDZ (Table 1). However, there was a decreased of the mean number of shoots recorded from 0.1 mg/L of TDZ to 0.2 mg/L of TDZ with mean of 2.95±1.39 and 2.10±0.25 respectively. The mean number of leaves recorded in Table 1 was varied from 5.20±1.52 to 7.95±1.22. As the concentrations increased from 0 mg/L of TDZ until 0.4 mg/L of TDZ, the mean number of leaves was generally increased. The control treatment had recorded the highest shoots which was 10.78±0.60cm. The shortest shoots were induced in 0.1 mg/L of TDZ with mean of 7.15±1.51cm.

The mean number of roots was recorded to increase as the concentration of TDZ increase until 0.2 mg/L of TDZ. Then, the number of roots was observed to decrease as TDZ concentration at 0.3 mg/L and 0.4 mg/L applied. The highest mean number of roots was induced in medium supplemented with 0.2 mg/L of TDZ which was 16.45±2.21. By using 0.2 mg/L of TDZ, thicker roots had been induced as compared to other treatments. As for other concentrations of TDZ, pale brown and slightly smaller in diameter of roots were produced especially when concentration of the hormone increased.

Tables 1 Mean (±SE) number of shoots, leaves, roots and height of shoots by using different concentrations of BAP, TDZ and KIN of *Curcuma caesia* Roxb.

Plant growth regulator (PGR)	Concentrations (mg/ L)	Mean (± SE)			
		No. of shoots	No. of leaves	Height of shoots (cm)	No. of roots
BAP	0	1.30±0.15	7.80±1.88	10.78±0.60	4.90±0.50a
	1.0	3.35±0.57	11.55±1.06	10.03±0.76	12.05±2.22b
	3.0	3.33±0.66	10.60±1.77	10.26±0.75	11.95±1.47b
	5.0	3.55±1.28	7.95±1.20	10.15±0.56	10.4±1.40ab
	8.0	4.00±0.82	9.75±1.22	9.96±0.52	8.85±0.44ab
	10.0	3.33±0.45	7.65±0.76	9.49±0.84	10.9±1.10b
TDZ	0	1.30±0.15	7.80±1.88	10.78±0.60	4.90±0.50a
	0.1	2.95±1.39	5.20±1.52	7.15±1.51	8.55±1.01ab
	0.2	2.10±0.25	7.50±1.82	7.80±1.21	16.45±2.21c
	0.3	2.25±0.37	7.95±1.22	9.84±0.76	13.3±1.31bc
	0.4	2.30±0.42	7.90±0.78	9.82±0.59	12.20±1.51bc
KIN	0	1.30±0.15	7.80±1.88	10.78±0.60	4.90±0.50a
	1.0	1.20±0.32	7.05±0.91	11.14±1.39	10.90±0.96bc
	2.0	1.20±0.36	7.30±0.81	12.52±0.92	12.50±1.08c
	3.0	2.34±0.52	10.05±1.38	12.08±0.73	14.75±1.87c
	4.0	2.10±0.43	10.90±1.20	12.55±0.72	12.90±1.22c

Mean (±SE) followed by same letters in a column are not significantly different at $p<0.05$ of Tukey test

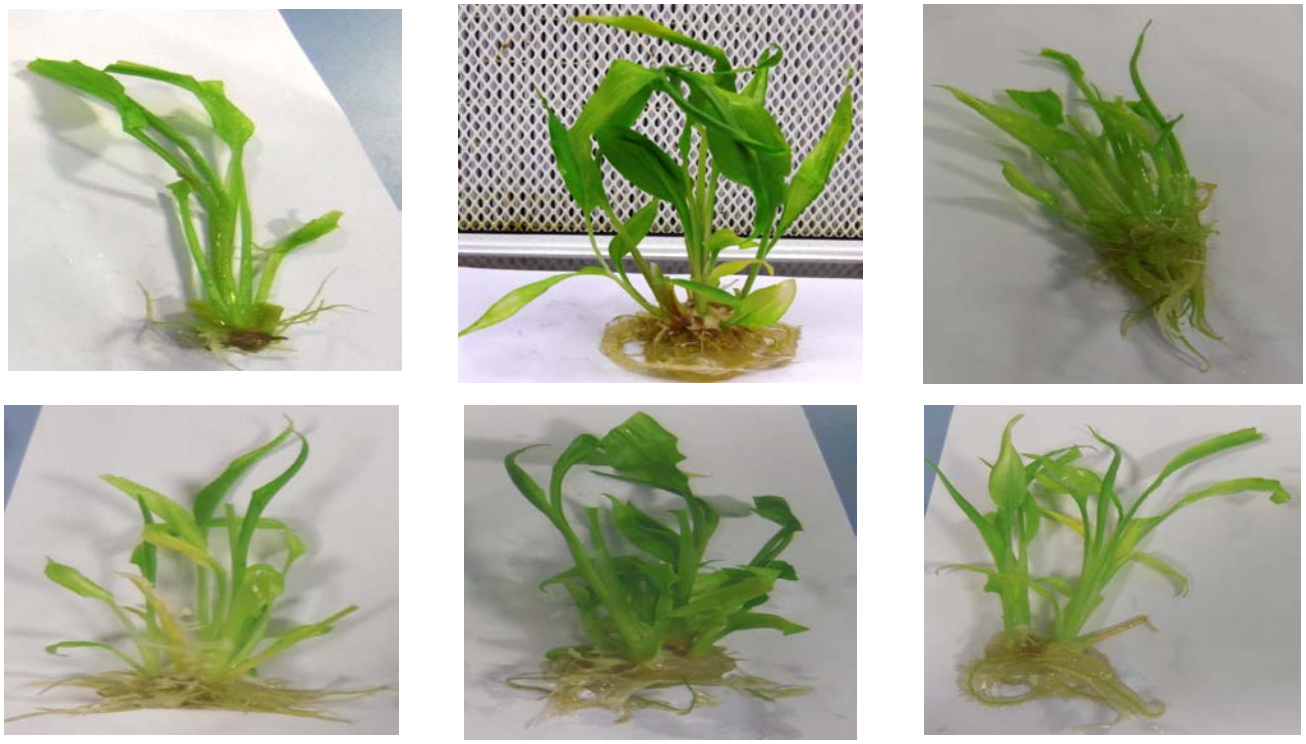

Figure 1: Multiple-shoot formation of *C. caesia* in MS medium supplemented with different concentrations of BAP after 12 weeks. a) Multiple-shoot formation of *C. caesia* in MS medium supplemented with 0 mg/L of BAP. b) Multiple-shoot formation of *C. caesia* in MS medium supplemented with 8.0 mg/L of BAP. c) Multiple-shoot formation of *C. caesia* in MS medium supplemented with 0.1 mg/L of TDZ. d) Multiple-shoot formation of *C. caesia* in MS medium supplemented with 0.4 mg/L of TDZ. e) Multiple-shoot formation of *C. caesia* in MS medium supplemented with 3.0 mg/L of KIN f) Multiple-shoot formation of *C. caesia* in MS medium supplemented with 4.0 mg/L of KIN. Scale bar is 0.5cm (a- d).

4.0 Discussions

The application of BAP alone (8.0 mg/L) in this study had recorded the highest number of multiple shoots among the plant growth regulators used. This result was further concurred with the findings of Bunnag *et al*. (2006) where the application of 5.0 mg/L of BAP had produced higher number of shoots in *Caulokaempferia thailandiica*. Bhosale *et al*. (2011) also reported that 7.0 mg/L of BAP had increased the average number of shoots in Banana varieties which were Ardhapuri, Basrai and Shrimanti.

The highest shoots multiplication in *Musa acuminate* cv. Berangan was also reported in medium supplemented with 33µm of BAP (Jafari *et al*., 2011). However, in a study conducted by Rao *et al*. (2011), 2.0 of mg/L and 3.0 of mg/L of BAP had been chosen as the best hormones used to induce shoots from *Alpinia galangal*. Balachandran *et al*. (1990) also reported that average of 3 shoots per explants were obtained for *C domestica*, *C. caesia* and *C. aeruginosa*, respectively in medium applied with BAP at 3.0 mg/L.

In the next sub-experiment using TDZ, the highest mean number of shoots was obtained in 0.1 mg/L of TDZ. TDZ was a cytokinin which initially synthesized for defoliation of cotton (Arndt et al., 1976). In this present observation, low concentrations of TDZ did not show any equal effect in inhibiting shoot elongation. However, shorter shoots were produced by using TDZ as compared to other types of hormones used in this experiment. This was further supported by Nieuwkerk et al. (1986) and Hamirah et al. (2009) where the application of TDZ in *Zingiber montanum* and *Malus domestica*, respectively had produced shorter plantlets. The dwarf plantlets of L. corniculatus were also obtained in medium incorporated with the lowest concentration of TDZ (Nikolic et al., 2006).

In the experiment of using KIN, medium supplemented with 3.0 mg/L of KIN had recorded the highest mean number of shoots (2.34±0.52) which was less

satisfied compared to BAP. This result was further similar with the finding of Borthakur *et al*. (1999) where 3.0 mg/L of KIN could induce high number of new shoots. Overall, the KIN hormone was not the ideal cytokinin for shoot induction of *Curcuma caesia*. The variation in plant response to different types of plant growth regulator was due to different endogenous hormone levels in the explants (Bhojwani & Razdan, 1996).

Based on all the results obtained, the experiment using BAP alone was selected as the best experiment to produce shoots for *Curcuma caesia*. Thus, 8.0 mg/L of BAP was recorded as the best concentration. However, Reddy *et al*. (2014) had reported that high concentration of BAP in the medium was not crucial for shoot production as it could increase the abnormality of the plantlets. Besides that, long term of application of high concentrations of BAP also had been observed to prevent the recovery of explants in tissue culture in becoming complete normal plants due to habituation effect of BAP (Reddy *et al.*, 2014). Hence, BAP at 1.0 mg/L could be chosen as the best concentration to replace 8.0 mg/L as the result in the BAP alone experiment showed no significantly difference between all the treatments. The 1.0 mg/L of BAP was chosen because of economic feasibility purposes and similar result to 8.0 mg/L of BAP.

5.0 Conclusion

The present study had shown that the best concentration of new shoots multiplication of *C. caesia* was 8.0 mg/L of BAP. However, for economic feasibility purposes, 1.0 mg/L of BAP which had recorded similar shoots as 8.0 mg/L of BAP was preferred.

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