

Clonal Micropropagation of Patumma (*Curcuma alismatifolia* Gagnep)

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ABSTRACT

Patumma is a new ornamental crop with high demand for export. The species is now vulnerable to extinction. Cloning methodology is therefore needed to produce plants for export and to conserve the germplasm. Young inflorescence and rhizome could be used as source of the lateral bud explant. The rhizome had to be air-dried for a week before used. Pre-treatment in 52°C water for 5-10 minutes greatly reduced the bacterial contamination. Plantlets were multiplied on modified MS media with 0, 6.67, 13.32, 19.98 and 26.64 $\mu\text{mol.l}^{-1}$ benzyladenine (BA) or 0.19, 0.56, 1.67 and 5.0 $\mu\text{mol.l}^{-1}$ kinetin. The maximum multiplication rate of 4.83 folds was obtained when longitudinally divided rhizome was cultured on the medium with 13.32 mmol.l^{-1} BA. The result also showed that wild-collected and selected clones responded to the media similarly. When the media modified with 13.32, 15.54, 17.76 and 19.98 $\mu\text{mol.l}^{-1}$ BA in combination with 15, 30 and 45 g.l^{-1} sucrose were tested, the multiplication rate of plantlets were all the same.

Key words : micropropagation, tissue culture, patumma, *Curcuma alismatifolia*

INTRODUCTION

Patumma (*Curcuma alismatifolia* Gagnep) is a native species of north-eastern Thailand. Its long-lasting colorful bract makes the plant suitable for cut-flower, potted plant and bedding plant. Due to an increasing demand of the rhizome, the wild plant is vulnerable to extinction. To ensure a sufficient supply of cultivated rhizome in the international market, a protocol for clonal micro-propagation of patumma is thus needed.

For the subgenus *Eucurcuma*, Balachandran *et al.* (1990) reported a protocol for clonal propagation of 3 *Curcuma* species. Marashige and Skoog (1962) medium supplemented with 0, 0.2, 1 or 3 mg.l^{-1} benzyladenine (BA) and a combination of 1 mg.l^{-1} BA and 1 mg.l^{-1} kinetin were tested for

the multiplication stage. Their results showed that using 3 mg.l^{-1} BA led to the maximum propagation rate. Apavacharut (personal communication) on the other hand, suggested that 0.02 - 0.2 mg.l^{-1} kinetin should be used. For the subgenus *Paracurcuma*, this report is the first study on axenic propagation.

MATERIAL AND METHODS

1. Preparation of explants

Both young inflorescences at the stage with all bracts were tightly close and rhizomes were used as sources of explants. Pieces of the inflorescence consisted of 2-3 bracts were excised after disinfestation, whilst lateral buds were removed from the rhizome before surface sterilization.

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The inflorescence of 'Chiangmai' cultivar was rinsed in running water for 30 minutes before soaking in 70% ethanol for a few minutes. The segment was then transferred into 0.5% NaOCl and shaken for 15 minutes. Rinsing with sterilized water for 3 times was performed prior to placing the explant on a medium.

The rhizome of wild-collected plant was dug up and dried for a week prior to the bud excision. The bud was cut and rinsed with running water for 30 minutes. After that it was left in 52°C water for 5-10 minutes before dipping in 70% ethanol. Disinfection with 0.79% NaOCl for 10 minutes was performed. The bud was then washed in sterilized water and placed on a medium.

2. Establishment of plant material

The explant was cultured on the modified Murashige and Skoog (1962) (MS) medium with 22.2 mmol.l⁻¹ BA under 16.9 - 22.1 mmol.m⁻².s⁻¹ of continuous light ("Daylight" fluorescent) at 26±2°C. Plantlets could be obtained after 6-8 weeks. The plantlet was further multiplied in the same medium till the sufficient number for the following experiment was achieved. All root were removed from plantlets and the shoot was cut down to 1 cm before every subculture.

3. Cloning media

The effects of BA and kinetin concentrations was investigated. The MS medium with 3% sucrose was supplemented with 0, 6.67, 13.32, 19.98 and 26.64 mmol.l⁻¹ BA or 0.19, 0.56, 1.67 and 5.0 mmol.l⁻¹ kinetin. The first group of plantlets was placed horizontally after the plantlets were longitudinally divided. All cultures were incubated under 16.9 - 22.1 mmol.m⁻².s⁻¹ of "Daylight" fluorescent lamp continuously at 28±2°C. In this experiment, plants from both inflorescences and rhizomes were employed.

In the second experiment, the plantlets originated from rhizomes were cultured on modified MS media with combination of 13.32, 15.54, 17.76 and 19.98 mol.l⁻¹ BA and 1.5, 3.0 and 4.5% sucrose. The plantlets were vertically placed on the media. The cultures were incubated in the same environment as in the first experiment, except that the temperature was 2°C lower.

RESULTS AND DISCUSSION

Stage 1 of micropropagation, namely establishment stage was rather successful by the described method. Over 70 percent of explant was

Table 1 Number of shoots per plantlet produced after patumma explant was cultured for 1 month on MS media containing BA.

BA ($\mu\text{mol.l}^{-1}$)	Undivided explant		Divided explant	
	Chiangmai	wild	Chiangmai	Wild
0	1.00	1.33	2.67	2.67
6.67	1.67	2.67	3.33	4.33
13.32	3.33	3.67	4.67	5.00
19.98	1.33	2.67	3.00	3.33
26.64	2.67	3.33	2.33	2.67
Significance				
Average	2.05	2.84	3.33	3.83

free from visible contamination and capable to grow. Both inflorescence segments and lateral buds from rhizomes appeared to be equally suitable for culture initiation. Thus, the rhizome should be dug up to get an explant only if necessary.

In the first experiment, the plantlet of both genotypes similarly responded the media (Table 2, 4). Undivided plantlets of the wild collected clone however performed better than that of 'Chaingmai' clone. Shoot multiplication of the plantlets was affected by the BA concentration, quadratically. The best result was obtained when 13.32 $\mu\text{mol.l}^{-1}$ BA was used (Table 1). Higher concentration caused hyperhydricity on the new shoot although more shoots were formed. The hyperhydric shoot was later dead. The higher multiplication rate of 4.83 folds in 6 weeks resulted when the divided plantlets were cultured on the best medium. This rate was higher than the multiplication rate of other *Curcuma spp.* on the same medium (4.05 folds) reported by Balachandran *et al.* (1990).

The sensitivity of plantlet to kinetin concentration was greater with divided plantlet than with undivided one (Table 3). It was possible due to the kinetin was absorbed more easily through cut-surface of the plantlet. With undivided plantlet, the

Table 2 Analysis of variance on number of patumma shoot from explant on the modified MS media with BA.

SOV	Significance
Undivided explant	
variety	L*
media	
non vs BA	L**
among BA	C**
Divided explant	
variety	ns
media	
non vs BA	L**
among BA	L**Q**

ns,*,** Not significant, significant at 5% level and 1% level, respectively.

L = linear, Q = quadratic, C = cubic

multiplication rate in all media were statistically equal (1.54 folds). The divided plantlet, on the other hand, showed a quadratic response to kinetin concentration. The highest multiplication rate of 4.5 folds was obtained on a medium containing 1.67 mmol.l^{-1} kinetin. It should be noted that the

Table 3 Number of shoots per plantlet produced after patumma explant was cultured for 1 month on MS media containing kinetin.

Kinetin ($\mu\text{mol.l}^{-1}$)	Undivided explant		Divided explant	
	Chiangmai	Wild	Chiangmai	Wild
0	1.00	1.33	2.67	2.67
0.19	1.33	1.00	3.00	4.00
0.56	1.67	2.00	4.00	4.00
1.67	1.33	1.33	4.67	4.33
5.00	1.67	2.00	2.33	3.33
Average	1.50	1.58	3.50	3.92

Table 4 Analysis of variance on number of patumma shoots from explant on the modified MS media with kinetin.

SOV	Significance
Undivided explant	
variety	ns
media	ns
Divided explant	
variety	ns
media	
non vs BA	L**
among BA	Q**

ns, Not significant, **significant at 1 % level.

L = linear, Q = quadratic

Table 5 Effects of BA and sucrose concentration on multiple rate and leaf growth of patumma cultured on modified MS media for 1 month.

Sucrose (%)	BA ($\mu\text{mol.l}^{-1}$)			
	13.32	15.54	17.76	19.98
1.5	2.17 ¹	2.00	2.83	3.17
	4.55	5.48	6.62	3.43
3.0	3.50	2.50	2.33	2.17
	7.80	6.52	7.83	5.93
4.5	3.67	3.33	3.00	3.17
5.27	6.38	6.52	3.70	

¹ Top line : Number of shoots per plantlet.

Bottom line : Average leaf length.

best concentration of BA and kinetin induced approximately equal multiplication rate with divided plantlet, but the newly formed shoot was thinner on kinetin containing medium.

In the second experiment, the tested concentration of BA and sucrose showed similar influence on multiple shoot production and leaf length (Table 5). But, it was observed that the higher the sucrose concentration, the better the root growth. This agreed with previous report (Balachandran *et al.*, 1990) reported that root proliferation in *Curcuma spp.* was not affected by the concentration of BA in the medium. Since sucrose concentration affected root growth, the manipulation on this factor should take an account for cloning operation. The medium with 13.21 mmol.l^{-1} BA and 3% sucrose was suitable for a single medium procedure. Plantlets from this medium were ready for deflasking.

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