Low Concentration of Paclobutrazol Induced Multiple Shoot and Plantlet Formation in Amethyst Curcuma

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บทคัดย่อ

จำนวนของตายอดเกิดขึ้นมากที่สุดเมื่อเพาะเลี้ยงตุ่มยอดจากเหง้าของกระเจียวฉัตรทิพย์ (*Curcuma* sp. var. *chattip*) บนอาหารพื้นฐานสูตร MS ซึ่งเติม 2,4-D 1 มิลลิกรัมต่อลิตร และน้ำมะพร้าว 15% ในขวดแก้วเป็นเวลา 4 สัปดาห์ ตายอดเหล่านี้สามารถเจริญต่อไปเป็นยอดและพืชจำนวนมากบนอาหารพื้นฐานสูตร MS ที่มี NAA 0.1 มิลลิกรัมต่อลิตร และน้ำมะพร้าว 15% พร้อมด้วย BA ความเข้มข้นแตกต่างกัน ภายใน 6 สัปดาห์ เมื่อเพาะเลี้ยงชิ้นส่วนปลายยอดบนอาหารพื้นฐานสูตร MS ซึ่งประกอบด้วย BA 5 มิลลิกรัมต่อลิตร NAA 0.1 มิลลิกรัมต่อลิตร และ paclobutrazol หลายความเข้มข้น โดยมีหรือไม่มีน้ำมะพร้าว 15% พบว่าจำนวนการเกิดต้นอ่อนสูงที่สุด (7.25 ต้นต่อชิ้นส่วนพืช) บนอาหารพื้นฐานสูตร MS ซึ่งเติม BA 5 มิลลิกรัมต่อลิตร NAA 0.1 มิลลิกรัมต่อลิตร paclobutrazol 0.01 มิลลิกรัมต่อลิตร และน้ำมะพร้าว 15% ภายหลัง 8 สัปดาห์

คำสำคัญ: กระเจียวฉัตรทิพย์ การเกิดต้นอ่อน การเพาะเลี้ยงเนื้อเยื่อพืช สารหน่วงการเติบโต

Abstract

The highest number of multiple shoot buds were obtained when cultured shoot sprouts from rhizome of amethyst curcuma (*Curcuma* sp. var. *chattip*) on MS basal medium supplemented with 1 mg/l 2, 4-D and 15% (v/v) coconut water in vitro for 4 weeks. These shoot buds could develop further into multiple shoots and plantlets on MS basal medium consisting of 0.1 mg/l NAA and 15% (v/v) coconut water in combination with different concentrations of BA within 6 weeks. After the culture of shoot tip explants on MS basal medium comprising 5 mg/l BA, 0.1 mg/l NAA and various concentrations of paclobutrazol with or without 15% (v/v) coconut water, the maximum number of plantlets formed (7.25 per explant) were found on MS basal medium fortified with 5 mg/l BA, 0.1 mg/l NAA, 0.01 mg/l paclobutrazol and 15% (v/v) coconut water 8 weeks afterward.

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1. Introduction

In Thailand, there are 2 well-known groups of ornamental *Curcuma*: Eucurcuma (krajeaw group in Thai) and Paracurcuma (patumma group in Thai). These perennial rhizomatous herbs are belonging to the Zingiberaceae family and originating from tropical and subtropical areas of northern Thailand and Cambodia [1], [2]. Amethyst curcuma (*Curcuma* sp. var. *chattip*), the Eucurcuma group, has recently been turned out to be attractive as a decorative flowering plant because of its beautiful violet-pink coma bracts of the compact spike. However, this monocotyledonous species has low rate of rhizome multiplication and some serious diseases, especially soft rot and bacterial wilt. Thus, vegetative reproduction of amethyst curcuma through tissue culture could be one of the appropriate ways to solve not only slow propagation rate but also prevent pathogenic infection.

Until now, many species of *Curcuma* had been cultured in vitro in various aspects, such as micropropagation and transformation of Siam tulip (*Curcuma alismatifolia*) [3]-[5], microrhizome production, plantlet regeneration and curcumin production in mango ginger (*C. amada*) [6], [7], aseptic propagation of tikhur (*C. angustifolia*) [8], microrhizome induction and somaclonal variation of *C. aromatica* [9], [10], callus-mediated shoot regeneration of *C. kwangsiensis* [11], [12], clonal multiplication and acclimatization of turmeric (*C. longa*) [13], [14], and zedoary or white turmeric (*C. zedoaria*) axenic propagation and callogenesis [15], [16]. Conversely, tissue culture of Eucurcuma group, especially, amethyst curcuma had never been reported. Therefore, the aim of this research was to investigate the effect of plant growth regulators (2,4-D and BA) and plant growth retardant (paclobutrazol: PBZ) on multiple shoots and plantlets formation of amethyst curcuma.

2. Materials and Methods

2.1 Surface Sterilization of Plant Materials

Shoot sprouts 1 cm long from healthy rhizome of amethyst curcuma (*Curcuma* sp. var. *chattip*) were collected and used as source of explants. They were washed thoroughly in running tap water and 5% (v/v) detergent solution Teepol for 10 min followed by rinsing in tap water for 2 min. Subsequently, explants were surface sterilized two times with Clorox (a commercial bleach solution comprised 5.25% (w/w) sodium hypochlorite as available chlorine), first with 10% (v/v) for 10 min and then 5% (v/v) for 10 min. The sprouts were afterward, rinsed 3 times with sterile distilled water (1 min each time).

2.2 Multiple Shoot Bud Formation

The aseptic shoot sprouts were trimmed to remove excess tissue and placed on MS basal medium [17] supplemented with 0.5, 1 and 2 mg/l 2,4-D individually or 0.5 mg/l 2,4-D in combinations with 0.1, 0.5 and 1.0 mg/l BA. All media were solidified with 2.4 % (w/v) gelrite and adding 4% (w/v) sucrose and 15% (v/v) coconut water (CW) as carbon source and organic supplement, respectively. The pH of each medium was adjusted to 5.6 using 0.1 N NaOH or HCl and autoclaved at 121°C for 20 min. All cultures were kept at 16 hr photoperiod with 18.24 μmol m^{-2} s^{-1} light intensity at 25 ± 1°C.

2.3 Multiple Shoot and Plantlet Formation

When multiple shoot buds had been obtained from shoot sprout explants, they were transferred into MS basal medium consisted of 0.1 mg/l NAA, 4% (w/v) sucrose and 15% (v/v) CW in combinations with 1, 2, 3, 4 and 5 mg/l BA. Later, shoot of the plantlets were cut 1 cm long and moved into MS basal medium consisted of 5 mg/l BA, 0.1 mg/l NAA and 4% (w/v) sucrose in combinations with 0.01 and 0.1 mg/l PBZ with or without 15% (v/v) CW. All cultures were incubated at the conditions as mentioned above.

2.4 Statistical Analysis

The experimental design for all treatments was CRD (completely randomized design). The ANOVA (analysis of variance) was first conducted at the significance level of $P<0.05$ and then comparison of means by DMRT (Duncan's multiple range test) were employed at $P<0.05$.

3. Results and Discussion

Amethyst curcuma (*Curcuma* sp. var. *chattip*), an ornamental plant, was mostly found in North-Eastern of Thailand. Due to the attractive coma bracts, this herbaceous plant now becomes an economically important cultivated species in the country. Thus, the achievement in micropropagation of amethyst curcuma may be sufficient for the increasing requirement of this plant. The present works showed that shoot sprouts of amethyst curcuma could develop into multiple shoot buds rather than callus on MS basal medium fortified with 2,4-D alone or in combination with BA (Table 1) after 4 weeks of in vitro culture. This outcome was different from *Curcuma aromatica* and *C. kwangsiensis* because callus development of those species had been found before plant regeneration occurred [10], [12]. The highest percentage of multiple shoot bud formation of amethyst curcuma was achieved when explants had been cultured on MS basal medium consisted of 1 mg/l 2,4-D (Figure 1). Result indicated that this concentration of 2,4-D was the beneficial proportion to 15% (v/v) coconut water on establishment of multiple shoot buds. Adding BA may interfere the proper ratio of auxin/cytokinin for shoot bud development.

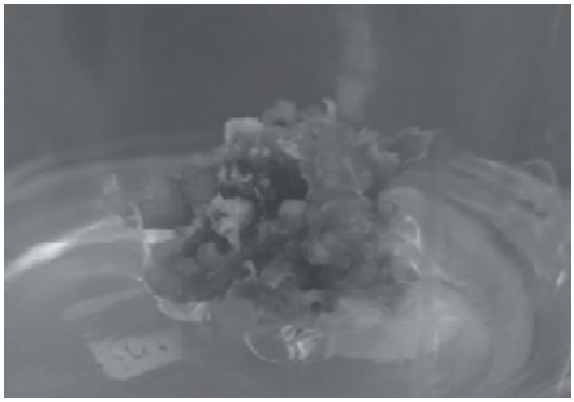

Figure 1 Multiple shoot bud formation of amethyst curcuma on MS basal medium consisted of 1 mg/l 2,4-D and 15% (v/v) CW.

Table 1 Percentage of multiple shoot bud formation from shoot sprouts of amethyst curcuma on MS basal medium containing 15% (v/v) CW with different concentrations of 2,4-D and BA

Plant growth regulator (s)	% multiple shoot bud formation
0.5 mg/l 2,4-D	53.4c
1.0 mg/l 2,4-D	78.2a
2.0 mg/l 2,4-D	56.7c
0.5 mg/l 2,4-D + 0.1 mg/l BA	49.86c
0.5 mg/l 2,4-D + 0.5 mg/l BA	66.67b
0.5 mg/l 2,4-D + 1.0 mg/l BA	61.34b

Values are means of 10 replications. Data marked by same letter in a column are not significantly different at $P < 0.05$

After transferred multiple shoot buds into MS basal medium consisted of 0.1 mg/l NAA, 15% (v/v) CW and various concentrations of BA for 6 weeks, it was revealed that the higher amount of BA gave the better number of multiple shoot formation and these shoots consequently developed into plantlets (Table 2). The maximum number of plantlet formation was observed on MS basal medium supplemented with 5 mg/l BA, 0.1 mg/l NAA and 15% (v/v) CW (Figure 2). As well as in *C. aromatica*, a concentration of 5 mg/l BA was also optimum for shoot multiplication [9]. This finding illustrated that growth and development of multiple shoots and plantlets of amethyst curcuma from multiple shoot buds demanded high concentration of BA. However, different species of *Curcuma* may need dissimilar amount of BA on shoot induction such as tikhur (*C. angustifolia*) which shoot initiation and elongation were obtained after shoot buds from rhizome were inoculated on MS medium supplemented with 3 mg/l BA [8].

Table 2 Number of plantlet formation of amethyst curcuma on MS basal medium comprised 0.1 mg/l NAA and 15% (v/v) CW in combination with various concentrations of BA

BA (mg/l)	Number of plantlet formation
1.0	1.2 e
2.0	1.8 d
3.0	2.5 c
4.0	3.2 b
5.0	5.1 a

Values are means of 10 replications. Data marked by same letter i a column are not significantly different at $P < 0.05$

From the previous works, paclobutrazol (PBZ) had been used for tissue culture of *Curcuma* in various ways. Kongbangkerd and Yanaphan [18] found that MS medium consisted of 0.1 mg/l PBZ was able to induce shoot of *C. longa* in vitro better than 0.5 mg/l. A number of shoots forming from this plant were 5.2 and 4.5, respectively. For Temulawak (*Curcuma xanthorrhiza*), Syahid [19] showed that MS medium supplemented with PBZ at 5 mg/l was capable of reducing plant growth without any abnormality after acclimatization in the glass house. Thus, PBZ could be applied to prolong in vitro conservation culture.

Figure 2 Amethyst curcuma plantlet formation on MS basal medium containing 5 mg/l BA, 0.1 mg/l NAA and 15% (v/v) CW.

Since our number of plantlet formation in MS basal medium consisted of 5 mg/l BA still had been unsatisfactory, low concentration of PBZ was tested in the present study whether it could help improving the plantlet quantity. Our results demonstrated that shoot tip explants cultured on MS basal medium consisted of 5 mg/l BA, 0.1 mg/l NAA and various concentrations of PBZ with or without 15% (v/v) CW could develop into multiple shoots and plantlets after 8 weeks (Table 3). The highest number of plantlet formation was noticed on MS basal medium fortified with 5 mg/l BA, 0.1 mg/l NAA, 0.01 mg/l PBZ and

Figure 3 Plantlet formation of amethyst curcuma on MS basal medium comprised 5 mg/l BA, 0.1 mg/l NAA, 0.01 mg/l PBZ and 15% (v/v) CW.

15% (v/v) CW (Figure 3). This number was higher than *C. longa* and *C. zedoaria* as the best average number of shoots per explant from those species were 6.7 and 5.6, respectively, when cultured the explant on Woody Plant Medium supplemented with 4 mg/l BA and 1.0 mg/l NAA and MS medium containing 20% (v/v) CW, 3 mg/l BA and 0.5 mg/l IBA, in that order [20], [16]. As a result, little amount of PBZ with 15% (v/v) CW apparently promoted multiple shoots and plantlets formation of amethyst curcuma better than none.

Table 3 Number of plantlet formation of amethyst curcuma on MS basal medium consisted of 5 mg/l BA, 0.1 mg/l NAA and different concentrations of PBZ with or without 15% (v/v) CW

Treatments	Number of plantlet formation
0.01 mg/l PBZ	3.00 d
0. 1 mg/l PBZ	5.00 b
0.01 mg/l PBZ + 15% CW	7.25 a
0.1 mg/l PBZ + 15% CW	3.75 c

Values are means of 10 replications. Data marked by same letter in a column are not significantly different at $P < 0.05$

In conclusion, though PBZ had been classified as a plant growth retardant, low concentration of this chemical seems to have a power to enhance multiple shoot and plantlet formation in some species of *Curcuma*, especially in amethyst curcuma. Nevertheless, it would be valuable to examine further whether PBZ is able to initiate in vitro flowering of amethyst curcuma since there was a report on inflorescence formation in feathered amaranth under aseptic conditions through this substance [21].

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