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Full Length Research Paper

Effects of plant growth regulators on callus induction from *Cananga odorata* flower petal explant

Z. Nurazah*, M. Radzali, A. Syahida and M. Maziah

Natural Product Laboratory, Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, University of Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

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The *Cananga odorata* callus was initiated from petals of the *C. odorata* flowers on MS medium and B5 vitamins containing 30 g/L sugar and 3 g/L agar. The medium was also supplemented with different concentrations of 1-naphtalene acetic acid (NAA), and combinations of NAA with 6-benzylaminpurine (BAP) as the plant growth regulators. It was observed that the *C. odorata* callus could be induced on media containing the combination of 3 mg/L NAA and 0.5 mg/L BAP. High concentration of NAA gave rise to pale, whitish and friable callus after 1 - 2 weeks of culture. The optimum pH for the cell culture was about 5.7 and incubation at 25 ± 2°C, in totally dark condition.

Key words: *Cananga odorata*, 1-naphtalene acetic acid, 6-benzylaminopurine, callus culture.

INTRODUCTION

Cananga odorata, which also commonly known as 'kenanga' or 'ylang-ylang', is a medium-size tree that has been introduced into many islands in the Pacific for its fragrant flowers (Burdock and Carabin, 2008). In Java, the dried flowers are used against malaria, and the fresh flowers are pounded into a paste to treat asthma (Duke, 2000). The high value of essential oil that is derived from *C. odorata* flowers is also widely used in aromatherapy. It said to have medicinal value by herbalists and aromatherapists (Manner and Elevitch, 2006). In Southeast Asia, the essential oil is used as a flavour in candies, icings, baked goods, chewing gum and candies (Burdock and Carabin, 2008).

Higher plants are a valuable source of wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives (Bourgaud et al., 2001). In the end of 1960s, plant cell culture technologies were introduced as a tool for both studying and producing plant secondary metabolites. A highly potent secondary metabolites that is used in pharmaceuticals and food additives have been produced through plant cell cultures, shoot cultures, root cultures and transgenic roots obtained through biotechnological means (Ramachandra Rao and Ravishankar, 2002). Callus or cell suspension culture culture also could be used for the large-scale plant cell culture where the bioactive compounds could be extracted (Taha et al., 2008).

So, this paper reports on the influence of plant growth regulators on induction and growth of *C. odorata* callus culture as a starting point to produce bioactive compounds in plant cell culture.

MATERIALS AND METHODS

Plant materials

C. odorata plants were regularly collected from Taman Pertanian Universiti (TPU), Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The voucher specimen (voucher no.: NZ01/09) was scientifically identified by a plant taxonomist, Mrs. Latifah Zainal Abidin and has been deposited at in the Herbarium of Faculty of Forestry, Universiti Putra Malaysia.

Explant preparation

C. odorata flower petal explants were washed under running tap water for 1 hour to remove dust and dirt, and soaked with mild detergent solution for 10 minutes. Then, they were washed three times with distilled water. The explants were surface sterilized and soaked in 70% (v/v) ethanol for 1 min and followed by 20% (v/v) sodium hypochlorite (containing one or two drops of 0.5% (v/v) Tween 20) for 10 minutes. These sterilized segments were then rinsed three times with sterile distilled water. The explants was cut into sections of 5 mm long and aseptically transferred onto a sterile

*Corresponding author. E-mail: nurazahzain@yahoo.com.

Table 1. Callus initiation from flower petal explant of *C. odorata* in the presence of various concentrations of NAA after 4 weeks of culture.

NAA (mg/L)	No. of explants produced callus (%) (n = 20)	Callus score (Mean ± SEM)	Texture
0.0	0 (0)	0.0 ± 0.00	-
1.0	4 (40)	0.4 ± 0.16	Friable
2.0	3 (30)	0.4 ± 0.22	Friable
3.0	6 (60)	0.8 ± 0.25	Friable

Notes: All cultures were incubated at 25±2°C, in completely dark condition. Twenty replicates were used for each treatment (n= 20).

glass Petri dish (9 cm diameter) for the short time before transferred to culture medium. All works were done under sterilized condition in a laminar air-flow cabinet.

Media and callus initiation

Flower petal explant was cultured on MS medium (Murashige and Skoog, 1962) and B5 (Gamborg, 1968) vitamins supplemented with various concentrations of 1-naphtalene acetic acid (NAA) (0.0, 1.0, 2.0 and 2.0 mg/L), and combinations of NAA and 6-benzylaminopurine (BAP) (Table 2). The pH for each medium was adjusted to pH 5.7, and was autoclaved at 121°C for 15 min. All cultures were incubated at 25 ± 2°C under completely dark condition. The callus growth was assessed between the first and fourth week. The pale and friable calluses were subcultured to a fresh medium every 4 weeks using the same conditions described above.

Growth measurement

Growth of callus was determined by fresh and dry weight measurement (for dry weight, callus was dried at 40°C for 2 days). Callus growth was also scored on the basis of amount, friability and texture of callus.

RESULTS AND DISCUSSION

Initially, callus was initiated from petal explant of *C. odorata* on MS medium and B5 vitamins supplemented with different concentrations of NAA (0.0, 1.0, 2.0 and 3.0 mg/L). Table 1 shows the *C. odorata* callus could be induced on medium supplemented with 1.0 mg/L up to 3.0 mg/L NAA, with different frequencies of callus induction and callus score. Friable, pale and white callus of *C. odorata* was grown in all concentrations of NAA that had been supplied. No callus was formed on medium without NAA. The highest callus score was obtained at concentration of 3.0 mg/L NAA, only after 4 weeks of culture with 60% of explants produced callus. Only little callus was formed with callus score 0.8 ± 0.25. Combination of NAA and BAP at various concentrations led to a faster growth of callus (Table 2). Callus was formed and grew well after two weeks of culture with different morphologies. The medium supplied with BAP alone as plant growth regulator gave rise to pale and white callus but compact in structure. Friable callus was obtained on medium supplied with combination of NAA (1.0 to 3.0 mg/L) and BAP (0.05 to 1.00 mg/L). At low concentrations of NAA (1 to 2 mg/L), in combination with BAP (0.05 to 1.00 mg/L), the callus obtained was reddish white to brown in colour and turned into pale and white in colour at high concentrations of NAA (3.0 mg/L).

It was found that a combination of high concentration of NAA and BAP had significant effects on callus formation. The maximum callus growth with callus score 2.0 ± 0.25 was obtained at concentration of 3.0 mg/L NAA and 0.50 mg/L BAP, with friable, pale and white callus (Figure 1a) with 100% callus induction frequency. On the contrary, treatment with high concentration of NAA (3.0 mg/L) and high concentration of BAP (1.00 mg/L) led the callus to turn brown. So, the best combination of 3.0 mg/L NAA and 0.50 mg/L BAP was used in proliferation of *C. odorata* petal-derived callus for further treatments. Callus grew well and remains proliferating (Figure 1b) even after 5 times of subculture. This suggests that higher concentration of NAA had influenced the callus growth and proliferation.

Several studies had been reported regarding the effects of plant growth regulators on callus growth of different plant species. Sirchl et al. (2008) found no callus formation from the seed explant of *Garcinia mangostana* on medium without NAA. According to Satyavathi et al. (2004), dicamba was the best plant growth regulator for callus induction from Durum wheat. Callus from Durum wheat was successfully induced at concentration of 2.0 mg/L dicamba. Kiong et al. (2008) also reported on callus induction from *Cycas revoluta*, where callus culture was formed on medium supplemented with 20 µM picloram. 0.5 mg/L 2,4-D supplemented medium had successfully induced the callus formation from *Gymnema sylvestre* (Gopi and Vatsala, 2006).

Combination of plant growth regulators also had successfully induced the formation of callus culture from many higher plants. *Leonurus heterophylus* callus culture was induced on MS medium supplied with combination of 2.0 mg/L BAP and 0.5 mg/L 2,4-D (Yang et al., 2008). Nirmalakumari (2006) also reported on callus induction from bark of *Vitex negundo* on medium supplemented with combination 2.5 mg/L 2,4-D and 0.75 mg/L kinetin. The formation of callus from *Vigna mungo* explant was induced on media containing 13.3 µM BAP and 13.5 µM

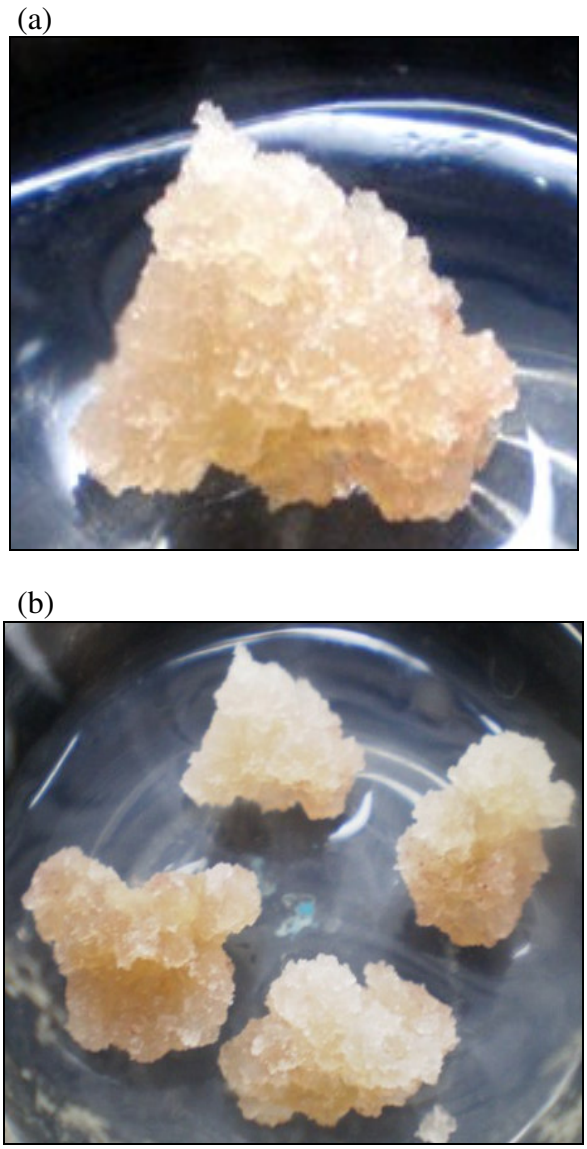

Figure 1 (a-b). A friable, pale and white *C. odorata* petal-derived callus on MS medium, B5 vitamins supplied with 3.0 mg/L NAA and 0.5 mg/L BAP at pH 5.7 at 25 ± 2 °C, in completely dark condition. (a) 4 weeks (b) 8 weeks of culture.

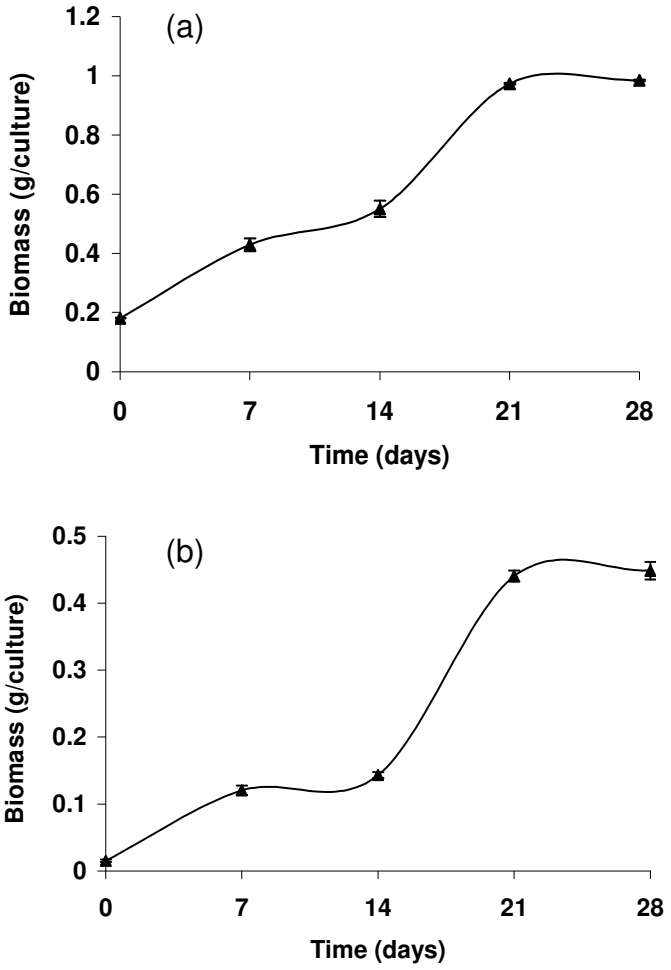

Figure 2 (a-b). Effect of 3mg/L plus 0.5 mg/L BAP supplemented medium on *C. odorata* petal-derived callus after 4 weeks of incubation time at pH 5.7, 25±2°C in completely dark condition. (a) Fresh weight and (b) dry weight.
Note: Data indicated mean ± standard deviation of triplicate determinations (n=3).

2, 4-D.

Growth of *C. odorata* callus was also determined by fresh and dry weight measurement. The fresh and dry weight of *C. odorata* petal-derived callus on medium supplemented with 3 mg/L NAA and 0.5 mg/L BAP were recorded every week until 4 weeks of cultivation time (Figure 2a-b). MS medium, B5 vitamins supplemented with 3.0 mg/L NAA and 0.5 mg/L BAP showed the highest biomass accumulation compared to other combinations of NAA and BAP (data not shown). Figure 2 (a-b) showed fresh weight (a) and dry weight (b) of *C. odorata* callus culture. It was observed that fresh and dry weights of callus were increased by cultivation time. There was a gradual increase in fresh and dry weights from day 14 with 0.55 ± 0.03 g/culture FW to day 21 with 0.97 ± 0.00 g/culture FW, and also the increment of dry weight from day 14 with 0.14 ± 0.00 g/culture DW to day 21 with 0.44 ± 0.01 g/culture DW. Day 28 showed the maximum biomass accumulation with 0.98 ± 0.00 g/culture fresh weight and 0.45 ± 0.01 g/culture dry weight. Other plant growth regulators treatments also had been done on callus induction from *C. odorata* (data not shown) but were terminated due to several problems such as browning, low callus induction frequency and compact structure of callus.

In conclusion, *C. odorata* petal-derived callus was successfully induced and maintained on MS medium and B5 vitamins supplemented with 3.0 mg/L NAA and 0.50 mg/L BAP with friable, pale and white callus. These findings provide some basic information for the production of bioactive compounds from *C. odorata* cell culture.

Table 2. Callus initiation from flower petal explant of *C. odorata* in the presence of various concentrations of PGRs: 1-naphtalene acetic acid (NAA) and 6-Benzylaminopurine (BAP) after 4 weeks of culture.

Combination of Plant Growth Regulators (PGRs) (mg/L)		No. of explants produced callus (%) (n = 20)	Callus score (Mean ± SEM)	Texture
NAA	BAP			
0	0.00	0 (0)	0.0 ± 0.00	-
0	0.05	7 (70)	1.0 ± 0.26	Compact
	0.10	4 (40)	0.4 ± 0.16	Compact
	0.50	8 (80)	1.2 ± 0.29	Compact
	1.00	8 (80)	0.8 ± 0.13	Compact
1	0.05	3 (30)	0.3 ± 0.15	Friable
	0.10	5 (50)	0.6 ± 0.22	Friable
	0.50	10 (100)	1.5 ± 0.17	Friable
	1.00	10 (100)	1.5 ± 0.17	Friable
2	0.05	10 (100)	2.1 ± 0.10	Friable
	0.10	9 (90)	1.1 ± 0.13	Friable
	0.50	10 (100)	2.1 ± 0.10	Friable
	1.00	10 (100)	1.6 ± 0.16	Friable
3	0.05	9 (90)	1.6 ± 0.27	Friable
	0.10	10 (100)	1.5 ± 0.22	Friable
	0.50	10 (100)	2.0 ± 0.25	Friable
	1.00	10 (100)	2.4 ± 0.16	Friable

Notes: All cultures were incubated at 25±2°C, in completely dark condition. Twenty replicates were used for each treatment (n=20).

Callus score (Muse, 1989): 0: no callus formed on explant, 1: little callus formed on explant, 2: callus formed at both sides of explant, 3: callus formed and covered the whole explant.

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