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Influence of Organic Supplements on Growth and Development of *In Vitro* Shoots of *Bulbophyllum dhaninivatii* Seidenf

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Abstract. In *vitro* shoot culture of *Bulbophyllum dhaninivatii* Seidenf. was conducted on semi-solid Vacin and Went (1949) medium supplemented with coconut water (50 100 and 150 ml/L), potato extract (25 and 50 g/L) and banana homogenate (25 and 50 g/L) for 12 weeks. The results found that the highest shoot number (6.92 shoots) could observe on the medium with 150 ml/L coconut water, 50 g/L potato extract and 50 g/L banana homogenate while the highest leaf and root number could obtain when cultured on the medium supplemented with 100 ml/L coconut water, 50 g/L potato extract and 50 g/L banana homogenate.

Introduction

Bulbophyllum is one of the largest genera in the Orchidaceae family. This genus comprised of about 3,000 species [1]. In Thailand around 210 species of the *Bulbophyllum* genus are now identified [2]. *Bulbophyllum dhaninivatii* Seidenf. was firstly found at Phu Miang Sub-District, Chatrakarn District, Phitsanulok Province. Nowaday, this species is also distributed in Phu Luang Wild Life Sanctuary, Loei Province and listed as an endemic species of Thailand [3]. Because of a limited distribution area and specific habitat, this species seems to become risk of extinction from natural site. *In vitro* propagation techniques have been widely used for conservation and succeeded in many orchid species i.e. in *Dendrobium* [4-7] and *Cymbidium* species [8-10] etc. *In vitro* culture and micropropagation *Bulbophyllum* species have been reported [11-14] and factors improving shoot multiplication in *Bulbophyllum* species, especially medium components, were also studied [15-17]. However, influence of other medium components, particularly organic supplements, on shoot regeneration efficiency of *Bulbophyllum* species has only few performed specifically in *B. dhaninivatii*. The objective of this study was conducted to test the effects of different concentration and combination of coconut water, potato extract and banana homogenate on shoot proliferation and plantlet regeneration potentials of *B. dhaninivatii in vitro*. Our receiving results will be useful for conservation planning and used as a model for *in vitro* conservation of other *Bulbophyllum* species.

Materials and methods

In vitro shoots of *B.dhaninivatii* Seidenf. from stock culture were transferred to culture on semi-solid Vacin & Went medium [18] supplemented with 20 g/L sucrose, 2.0 g/l activated charcoal and 7.5 g/L agar. A different concentrations of a combination between coconut water (CW) (50, 100, 150 mL/L), potato extract (PE) (25, 50 g/L) and banana homogenate (BH) (25, 50 g/L) were additionally filled in the medium. The pH of medium was adjusted to 5.2. The cultures were placed under 12 hrs photoperiod of LED warm white light with 20 $\mu mol.m^{-2}.s^{-1}$ PPF at 25-27 ºC. The data were collected at 12 weeks after inoculation for number of shoots, roots and leaves, shoot, root and leave length, width of leaves. Experimental design was conducted on 3x2x2 Factorial in Complete

Randomize Design. Each treatment consisted of 15 replicates. The experiment was repeated thrice. Data collected were subjected to analysis of variance and means were separated by Duncan's Multiple Range Test (DMRT).

Results and discussions

An organic supplements, one of an important medium component, have been used to stimulate growth, development and promote shoot regeneration in many orchid tissue cultures i.e. in *Dendrobium* [19], *Cymbidium pendulum* [20], *Vanda* and *Mokara* [21], *Vanda helvola* [22] and *Cypripedium macranthos* [23] etc. *In vitro* shoots of *B.dhaninivatii* were cultured on semi-solid VW medium supplemented with different concentrations of a combination between CW, PE and BH for 12 weeks. The results indicated that morphological change of *in vitro* shoots could be noticed after the second week of culture. Enlargements of explants with shoot bud induction as well as new root formation in all treatments were then observed after 4 weeks of culture. New regenerated shoots with green leaves were then investigated at 8 weeks and continuously grew into plantlets after 12 weeks of culture. The overall results found that adding organic supplements to the medium improved better growth and morphological change of *in vitro* shoots than that cultured on the control medium. The highest shoot regeneration number was observed on the medium supplemented with highest concentration of CW (150 mL/L), PE (50 g/L) and BH (50 g/L) (Table 1, Fig.1). No surprising results was noticed on this condition because CW contains several types of cytokinin [24] which could be promoted shoot multiplication in many orchids [25-26] included in *B.dhaninivatii* while PE and BH could also stimulate shoot growth and regeneration efficiency when combined with CW [20, 27]. The highest root and leaf number were obtained when maintaining the higher concentration of PE (50 g/L) and BH (50 g/L) with a reduction of CW to 100 ml/L. The related result has been observed in *Calanthe* hybrids [28]. In consideration of shoot, root and leaf growth in *B.dhaninivatii*, adding 50 ml/L CW with 25 g/l PE and 50 g/L BH to the medium promoted the highest shoot (3.59 cm) and root length (1.83 cm) as well as leaf width (0.58 cm) and length (2.87 cm), respectively. The relative effect of the suitable ratio between CW: PE: BH has been studied in *Vanda teres* [29], *Spathoglottis kimballiana* [30] and *Cymbidium pendulum* [20] etc.

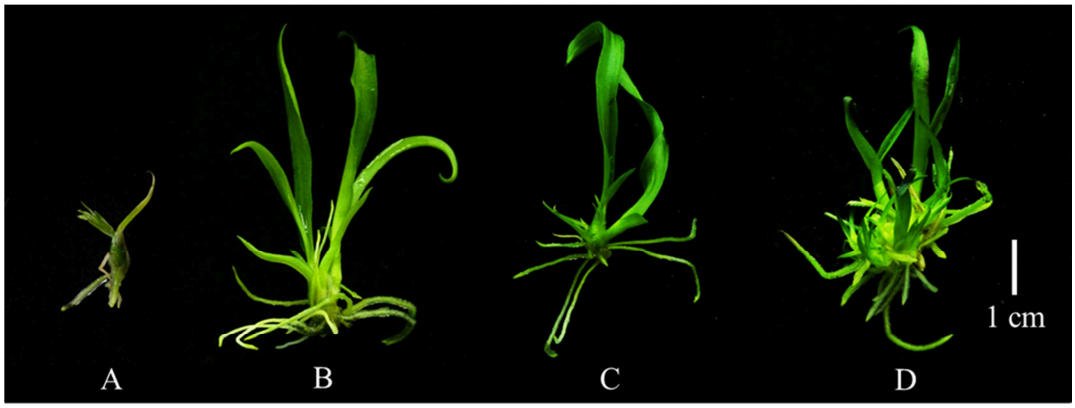

Fig.1 Plantlet after cultured for 12 weeks. (A) VW medium. (B) VW medium supplemented with 50 mL/L CW, 25 g/L PE and 50 g/L BH. (C) VW medium supplemented with 100 mL/L CW, 50 g/L PE and 50 g/L BH. (D) VW medium supplemented with 150 mL/L CW, 50 g/L PE and 50 g/L BH.

Table 1. Effect of coconut water (CW), potato extract (PE) and banana homogenate (BH) supplementation on growth of *in vitro* shoot of *Bulbophyllum dhaninivatii* after cultured for 12 weeks.

CW [mL/L]	PE [g/L]	BH [g/L]	Shoots		Leaves			Roots	
			Number	Length [cm]	Number	Length [cm]	Width [cm]	Number	Length [cm]
0	0	0	0.8 ± 0.2 b*	1.26 ± 0.30 d	1.0 ± 0.3 d	0.73 ± 0.23 d	0.10 ± 0.03 c	3.1 ± 0.8 c	0.55 ± 0.13 d
50	25	25	4.5 ± 1.4 ab	2.19 ± 0.44bcd	2.4 ± 0.3 ab	2.52 ± 0.48 ab	0.53 ± 0.25 ab	7.3 ± 1.2 abc	1.31 ± 0.20 abc
		50	4.1 ± 1.5 ab	3.59 ± 0.52 a	2.2 ± 0.3 abc	2.87 ± 0.37 a	0.58 ± 0.19 a	10.7 ± 2.1 ab	1.83 ± 0.22 a
	50	25	4.1 ± 1.5 ab	3.50 ± 0.39a	2.7 ± 0.4 ab	2.74 ± 0.29 a	0.27 ± 0.02 bc	9.5 ± 1.5 ab	0.96 ± 0.17 bcd
		50	3.8 ± 2.1 ab	3.07 ± 0.36abc	2.2 ± 0.3 abc	2.45 ± 0.31 abc	0.29 ± 0.02 abc	9.1 ± 1.3 ab	1.34 ± 0.18 abc
100	25	25	2.6 ± 0.5 b	3.15 ± 0.44 ab	2.8 ± 0.3 a	2.39 ± 0.36 abc	0.29 ± 0.03 abc	8.4 ± 0.8 ab	1.53 ± 0.17 ab
		50	2.1 ±0.4 b	1.97 ± 0.38cd	2.0 ± 0.3 abcd	1.53 ± 0.33bcd	0.24 ± 0.03 bc	9.3 ± 1.2 ab	1.45 ± 0.15 abc
	50	25	3.0 ± 1.1 b	2.04 ± 0.41bcd	2.5 ± 0.6 ab	1.36 ± 0.34 d	0.20 ± 0.04 c	8.3 ± 1.4 ab	1.04 ± 0.11 bcd
		50	2.3 ± 0.6 b	3.55 ± 0.38 a	2.9 ± 0.3 a	2.80 ± 0.26 a	0.34 ± 0.03 abc	12.5 ± 1.5 a	1.33 ± 0.21 abc
150	25	25	3.0 ± 0.8 b	1.68 ± 0.36 d	1.5 ± 0.4 bcd	1.19 ± 0.32 d	0.17 ± 0.04 c	6.6 ± 1.4 bc	1.03 ± 0.20 bcd
		50	3.0 ± 0.4 b	1.53 ± 0.22 d	1.7 ± 0.4 abcd	0.97 ± 0.29 d	0.15 ± 0.04 c	10.6 ± 2.3 ab	0.93 ± 0.13 cd
	50	25	4.4 ± 1.0 ab	1.95 ± 0.35 cd	1.7 ± 0.4 abcd	1.42 ± 0.34 cd	0.24 ± 0.06 bc	10.1 ± 2.8 ab	1.17 ± 0.19 bc
		50	7.0 ± 1.4 a	1.21 ± 0.16 d	1.1 ± 0.3 cd	0.98 ± 0.27 d	0.15 ± 0.04 c	9.5 ± 1.6 ab	1.24 ± 0.22 bc

*Values are means ± SD of 3 repeats (10 *in vitro* shoots per repeat). Different letters within the same column show highly significant differences analyzed by Duncan's new multiple range tests at $p \leq 0.05$.

Summary

From our investigation, the effects of different organic supplements on shoot regeneration and multiplication of *B.dhaninivatii* were studied and improved. The results revealed that adding 100 ml/L coconut water with 50 g/L potato extract and 50 g/L banana homogenate to the VW medium was the most influential for shoot regeneration and plantlet development. Organic supplements could promote shoot multiplication of *B.dhaninivatii* when compared to the control. Our study is an alternative way for rapid propagation of *B.dhaninivatii* without using plant growth regulators.

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