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In Vitro Propagation of *Spathoglottis* sp

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Abstract

This study aimed to find out which medium is suitable for *in vitro* cultivation of orchid *Spathoglottis* sp. by comparing the growth rate in two different media. The first experiment, the orchid seedlings were cultured in a modified synthetic formula of Murashige and Skoog (MS) medium (1962) added with plant growth regulators such as 6-benzylaminopurine (BA) combined with α -naphthaleneacetic (NAA) in 1 and 2 mg/l concentrations and adjusted pH to 5.8. The second experiment, the orchid seedlings were cultivated in a modified medium of Vacin and Went (VW) (1964) supplemented with potato, mashed banana and coconut water in various concentrations of 5, 100 g/l and 150 ml/l, respectively. All of the media were added with 20 g/l sugar, 8g/l agar and 2 g/l activated charcoal, and pH was adjusted to 5.0. All samples were cultivated under light intensity of 2,500 lux at 25 ± 2 °C for 16 h per day for 12 weeks. Results showed that the orchid seedlings cultured in MS medium and supplemented with plant growth regulators of 1 mg/l NAA gave higher average of shoot length, leaf length and root numbers (7.923, 4.303 and 7.843 cm, respectively). Orchids cultured in VW medium added with coconut water in 50 ml/l gave a high average of shoot length, leaf length, root numbers and root weight (8.980, 4.560, 4.733 and 0.637 cm., respectively). As a result, this study has revealed that the most suitable medium for *in vitro* propagation of *Spathoglottis* sp. was the MS medium that added with 2 mg/l of NAA.

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1. Introduction

Orchids are naturally and commercially flower and potted plants within floriculture trade worldwide. It is considered outstanding in the ornamentals due to its diverse colors, shapes, forms and long lasting blooms. Among the terrestrial orchid, *Spathoglottis* sp. is an occupy famous with marvellous varieties such as long grassy leaves, more than 8.0 cm wide flowers, and long time attractive blooming period which is admirable for purity of blooms and prolonged shelf life (Mukherjee, 2002). Many species were reported in the various national parks of Thailand including *Spathoglottis lobii*, *S. pubescens*, *S. plicata* (Thaithong, 1999). The multicultural orchid was propagated by seed, division of clumps, cuttings, separation of off-shoots. The major constraint in the conventional plant propagation is the slow in clonal multiplication. Therefore, *in vitro* tissue culture is used as an

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alternative method for rapid mass multiplication of the valuable varieties, in that it has progressed well during last decades and preferably has been used in recent years in developed countries.

Plant tissue culture technique is eventually an effective solution for the mass multiplication in limiting periods. Therefore, this study aimed to find out which medium was suitable for *in vitro* cultivation of orchid *Spathoglottis* sp. to establish the protocol for *in vitro* shoot proliferation and rooting of fascinating orchid. The *in vitro* propagation of *Spathoglottis* sp. is successfully carried out through the use of appropriate plant medium and growth regulators. The establishment of a reliable cloning methodology for this orchid is important in terms of enabling the rapid propagation and production of a large number of high quality plants.

2. Materials and method

This study aimed to find out which medium was suitable for *in vitro* cultivation of orchid *Spathoglottis* sp. Orchid seeds were harvested after 20 days of artificial pollination. Wash capsules of orchids in 10% clorox (NaOCl) added with 2 - 3 drops of Tween 20, shake for 10 min, then wash with sterilized distilled water three times, 5 minutes each. The capsules were dipped in 95% ethyl alcohol and flamed for three times. The sterilized capsules were cut longitudinally by a scalpel. Then the seeds were cultured in basic Vacin and Went medium for 4 weeks. Sub culture of protocorms was done at 3 weeks after inoculation to compare the growth rate in two different media. The first experiment, the orchid seedlings were cultured in a modified synthetic formula of Murashige and Skoog (MS) medium (1962) added with plant growth regulators such as 0.1 mg/l 6-benzylaminopurine (BA) combined with 2.0 mg/l α -naphthaleneacetic (NAA) and adjusted pH to 5.8. The second experiment, the orchid seedlings were cultivated in a modified medium of Vacin and Went (VW) (1964) supplemented with 50 mg/l potato, 100 mg/l banana and 150 mg/l coconut water. All of the media were added with 20 g/l sugar, 8 g/l agar and 2 g/l activated charcoal, and pH was adjusted to 5.0. All samples were cultivated under light intensity of 2,500 lux at 25 ± 2 ° C for 16 h per day for 12 weeks. The growth of seedlings were measured as plant height, leaf number, leaf size, root length and fresh weight and the results were analyzed statistically by using ANOVA. In both experiments, factorial in completely randomized design with 3 replicates was used. In experiment 1, Factor A was the levels of NAA (0,1 and 2 mg/l), and factor B was the levels of BA (0,1 and 2 mg/l). For experiment 2, factor A was the organic compounds supplemented in the medium (potato, banana and coconut water). And factor B was three levels (50, 100 and 150 mg/l) of those organic compound.

3. Results

After sub culture from modified VW medium into a modified medium of Vacin and Went, all samples were cultivated under light intensity of 2,500 lux at 25 ± 2 ° C for 16 h per day for 5 weeks. Seedlings of *Spathoglottis* sp. were developed into 5 stages. Stage I was the germination of seeds, seed coat ruptured by enlarged embryo after 2 weeks. Stage II, rhizoids (rz) were developed after 3 weeks. Stage III – IV were the presence of leaf primordium (lp) of embryo after 4 weeks and the appearance of the first true leaf (tl) after 5 weeks and stage V was elongation of initial leaves (Figure 1).

Experiment 1, the effect of Murashige and Skoog (MS) medium (1962) added with plant growth regulators such as 0.1 mg/l BA combined with 2.0 mg/l NAA was studied. The results found that the medium added 1 mg/l NAA could increase shoot length to 6.528 cm. The medium added with 2 mg/l NAA combined with 2 mg/l BA could produce leaves to 3.530 leaves/shoot. The medium added 1 mg/l NAA could extend leaf length to 7.848 cm. The medium added with 1mg/l NAA could increase the number of roots to 7.843 roots/shoot. The medium added with 2 mg/l NAA combined with 2 mg/l BA could increase the root length to 4.530 cm/root. The medium added with 1 mg/l NAA combined with 1 mg/l BA gave the highest weight of seedlings at 0.443 g/shoot (Table 1).

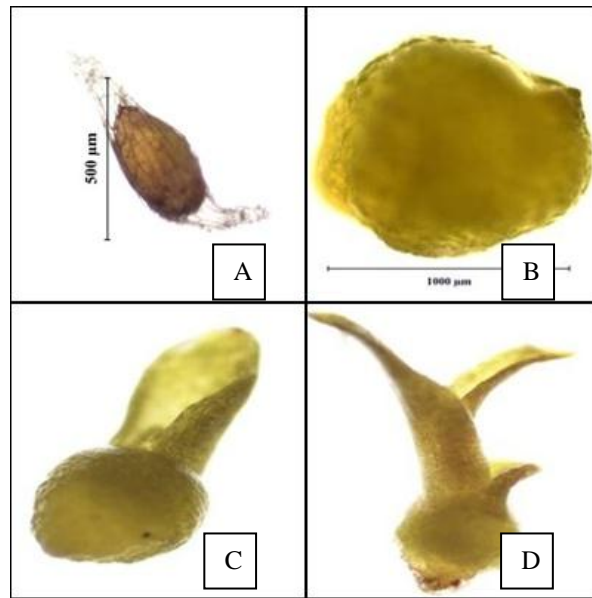


Fig. 1. Developmental stages of *Spathoglottis* sp. A. Nongerminated embryo B. Stage I = Germination C. Stage III-IV = The presence of leaf primordium (lp) and the appearance of the first true leaf (tl) D. Stage V = Elongation of initial leaves

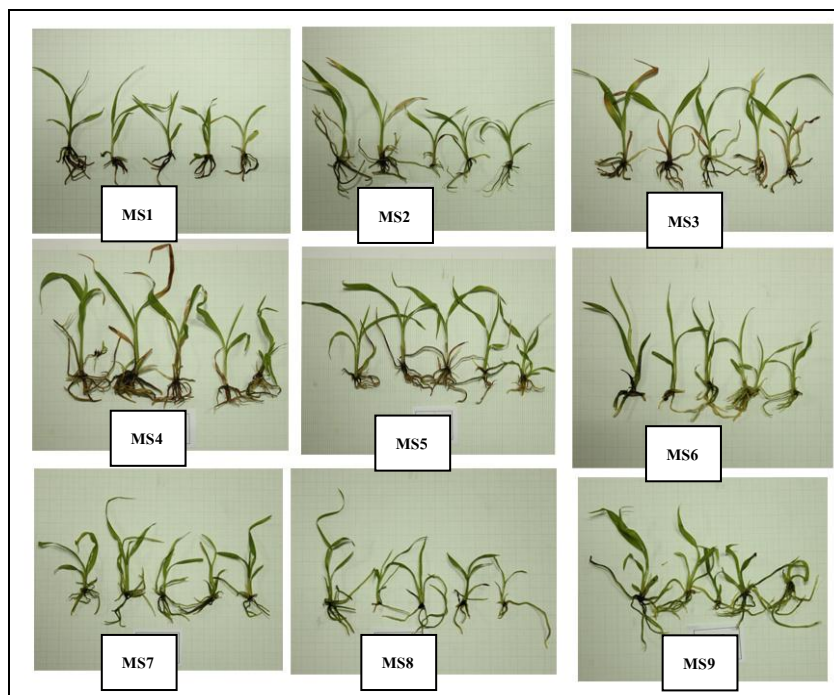


Fig. 2. *In vitro* propagation of *Spathoglottis* sp. in MS medium
Photo taken after 8 weeks of inoculation

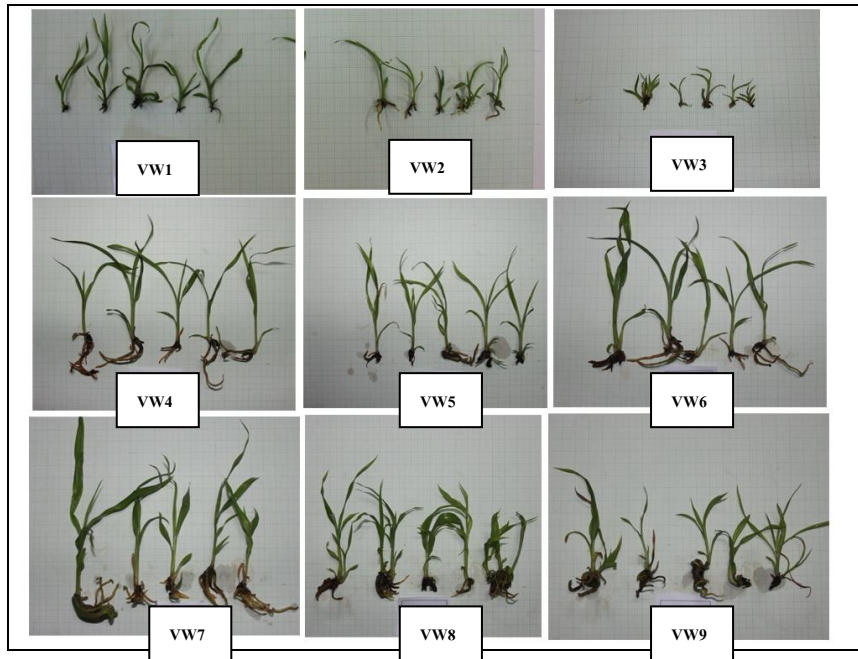


Fig. 3. In vitro *Spathoglottis* sp. in VW medium
Photo taken after 8 weeks of inoculation

Table 1. Growth of orchid seedlings cultivated in Murashige and Skoog (MS) medium (1962) added with 0.1 mg/l 6-benzylaminopurine (BA) combined with 2.0 mg/l NAA

Factors		Shoot length (cm)	Leaves/shoot	Leave length (cm)	Leave width (cm)	Root numbers (root/shoot)	Root length (cm)	Root weight (g)
NAA	0 mg/l	5.726 ^{ab}	2.963	3.070	0.231	4.760 ^b	1.951b	0.223 ^b
	1 mg/l	6.528 ^a	3.079	3.448	0.234	6.959 ^a	2.820 ^{ab}	0.343 ^a
	2 mg/l	4.378 ^b	3.183	2.174	0.227	5.479 ^b	3.468 ^a	0.198 ^b
F-test		**	ns	ns	ns	**	*	*
BA	0 mg/l	5.966	3.058	3.220	0.211	5.790	2.369	0.256
	1 mg/l	5.591	3.104	3.097	0.258	5.848	2.752	0.267
	2 mg/l	5.070	3.063	2.376	0.223	5.560	3.118	0.242
F- test		ns	ns	ns	ns	ns	ns	ns
MS + NAA 0 + BA 0 mg/l		5.713	3.087 ^{ab}	3.183 ^{bac}	0.210	4.507 ^c	1.587	0.163
MS + NAA 0 + BA 1 mg/l		5.387	3.163 ^{ab}	2.720 ^{bc}	0.237	4.620 ^{bc}	2.250	0.197
MS + NAA 0 + BA 2 mg/l		6.063	2.640 ^b	3.307 ^{ab}	0.247	5.153 ^{bc}	2.017	0.310
MS + NAA 1 + BA 0 mg/l		7.923	3.087 ^{ab}	4.303 ^a	0.197	7.843 ^a	2.760	0.400
MS + NAA 1 + BA 1 mg/l		6.897	3.130 ^{ab}	4.340 ^a	0.303	7.727 ^a	2.893	0.443
MS + NAA 1 + BA 2 mg/l		4.763	3.020 ^{ab}	1.700 ^c	0.203	5.307 ^{bc}	2.807	0.187
MS + NAA 2 + BA 0 mg/l		4.260	3.000 ^{ab}	2.173 ^{bc}	0.227	5.020 ^{bc}	2.760	0.203
MS + NAA 2 + BA 1 mg/l		4.490	3.020 ^{ab}	2.230 ^{bc}	0.233	5.197 ^{bc}	3.113	0.160
MS + NAA 2 + BA 2 mg/l		4.383	3.530 ^a	2.120 ^{bc}	0.220	6.220 ^b	4.530	0.230
F- test		ns	*	*	ns	**	ns	ns

ns = not significant

Means followed by the same letters in the column are not significantly different according to DMRT at $p < 0.05$ (*) and $P < 0.01$ (**)

Experiment 2, the results showed that modified medium of Vacin and Went supplemented with mashed potato and banana increased the height of shoot. 50 g/l of mashed banana could increase the average length of shoot to 7.40 cm. In addition, it also found that banana and coconut water produced more numbers of leaves. 150 ml/l coconut water gave the highest number of leaves (3.80 leaves/shoot). The medium added with 50 ml/l coconut water gave the length and the width of leave at 4.560 and 0.340 cm., respectively, and the number of roots at 4.733 root/shoot, the weight of fresh shoot at 0.673 g/shoot. 150 ml/l coconut water could arouse the length of root at 2.983 cm. (Table 2)

Table 2. Growth of orchid seedlings cultivated in a modified medium of Vacin and Went (VW) (1964) supplemented with 50 mg/l potato, 100 mg/l banana and 150 ml/l coconut water

Factors	Shoot length (cm)	Leaves/shoot	Leave length (cm)	Leave width (cm)	Root number (root /shoot)	Root length (cm)	Root weight (g)
Potato (P)	2.89 ^b	3.178 ^b	1.987 ^b	0.324	1.457 ^b	0.578 ^b	0.049 ^c
Banana (B)	7.151 ^a	3.613 ^a	3.617 ^a	0.304	3.768 ^a	1.571 ^a	0.251 ^b
coconut water (CW)	7.367 ^a	3.567 ^a	4.029 ^a	0.319	4.234 ^a	2.2234 ^a	0.466 ^a
F-test	**	*	**	ns	**	**	**
volume 50 g/l	7.401 ^a	3.197	3.731 ^a	0.348	3.547	1.552	0.331 ^a
100 g/l	5.166 ^b	3.600	3.254 ^{ab}	0.327	2.877	1.331	0.217 ^b
150 ml/l	4.849 ^b	3.561	2.647 ^b	0.273	3.036	1.600	0.218 ^b
F- test	**	ns	*	ns	ns	ns	**
VW + P 50 g/l	4.443	3.043	2.357	0.373	1.953	0.680 ^{cd}	0.057 ^e
VW + P 100 g/l	2.500	3.360	2.727	0.353	1.640	0.813 ^{cd}	0.050 ^e
VW + P 150 g/l	1.750	3.130	0.867	0.247	0.777	0.240 ^d	0.040 ^e
VW + B 50 g/l	8.780	3.400	4.277	0.330	3.953	1.793 ^b	0.300 ^{bcd}
VW + B 100 g/l	6.390	3.687	3.263	0.293	3.107	1.343 ^{bc}	0.253 ^{cd}
VW + B 150 g/l	6.283	3.753	3.310	0.290	4.243	1.577 ^{bc}	0.200 ^d
VW + CW 50 ml/l	8.980	3.147	4.560	0.340	4.733	2.183 ^{ab}	0.637 ^a
VW + CW 100 ml/l	6.607	3.753	3.763	0.333	3.883	1.537 ^{bc}	0.347 ^{bc}
VW + CW 150 ml/l	6.513	3.800	3.763	0.283	4.087	2.983 ^a	0.413 ^b
F- test	ns	ns	ns	ns	ns	**	*

ns = not significant different

Means followed by the same letters in the column are not significantly different according to DMRT at $p < 0.05$ (*) and $P < 0.01$ (**)

4. Discussions

The results showed that the orchid cultured in modified MS medium and supplemented with plant growth regulators could grow well. Adding 1 mg/l NAA could increase shoot length to 6.528 cm. The medium added with 2 mg/l NAA combined with 2 mg/l BA produced the maximum number of leaves at 3.530 leaves/shoot. The medium added with 1 mg/l NAA gave the maximum leaves length at 7.848 cm/leaf and the number of roots at 7.843 roots/shoot. The medium added with 2 mg/l NAA combined with 2 mg/l BA increased the root length to 4.530 cm. The medium added with 1 mg/l NAA combined with 1 mg/l BA had the highest root weight at 0.443 g/shoot. The combinations of NAA and BA showed the significance in the production of the multiple leaves, leave length and root numbers. Talukdar and Ahmed (2003) reported the highest seed germination percentage, growth and protocorm development on Knudson C medium supplemented with 50 ml/l coconut water, 0.5 g/l ME, 0.5 mg/l kinetin, 0.5 mg/l IAA and 50 ml/l tomato juice. The results of this study showed that modified medium of Vacin and Went (VW) added with mashed potato and banana affected on the length of shoot. Mashed banana could increase the length of shoot at 7.40 cm. 150 ml/l coconut water could produce the number of leaves at 3.80 leaves/shoot. The medium added with 50 ml/ 1 coconut water gave the length and the width of leave at 4.560 and 0.340 cm/leave, respectively, the number of roots at 4.733 root/shoot and the weight of fresh shoot at 0.673 g/shoot. 150 ml/l coconut water could arouse the length of root at 2.983 cm. The result of this study was in according with the study of Mao Minea et al. (2002) showing the effects of paclobutrazol and blended banana to promote the seedling growth. In summary, the optimum plant growth regulator combination for modified MS medium for *Spathoglottis* sp. was 1 mg/l NAA. For modified VW medium, adding media with 50 ml/l coconut water was the best combination for *Spathoglottis* sp. in this study.

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