Hue University Journal of Science: Agriculture and Rural Development ISSN 2588–1191
Vol. 126, No. 3E, 2017, P.13–19 ;
DOI: 10.26459/hueuni-jard.v126i3E.4425

IN VITRO PROPAGATION OF FINLAYSON'S CYMBIDIUM ORCHID

Bui Quoc Minh, Phan Thi Phuong Nhi*

HU – University of Agriculture and Forestry, 102 Phung Hung St., Hue, Vietnam

Abstract: Finlayson's Cymbidium is a native species in Vietnam, and this is one of the orchids favorite for many orchidists. In order to enrich this species, the propagation was conducted *in vitro*. The experiments were carried out to germinate protocorm of orchid seeds, multiply shoots and develop the complete plant formation. The experiments were organized in a completely randomized design with three replications for each test medium. The results showed that the MS medium supplemented with 20 g/L saccharose, 6 g/L agar, 1 g/L activated charcoal, 10 % coconut water and 1 mg/L 6-benzyladenine was the optimal medium for protocorm shooting. In the shoot multiplication stage, the MS medium supplemented with 1 g/L activated charcoal, 20 g/L saccharose, 6 g/L agar and 2 mg/L 6-benzyladenine was the most suitable. For rooting and developing plant formation, the suitable medium was MS supplemented with 20 g/L saccharose, 6 g/L agar, 1 g/L activated charcoal and 0.7 mg/L 1-naphthaleneacetic acid. The highest 2.6 roots and 3.8 leaves per plant were found. The plant height was 5.34 cm after the 6-week culture.

Keywords: Cymbidium, *in vitro*, shoot multiplication

1 Introduction

Finlayson's Cymbidium (*Cymbidium finlaysonianum* Wall. ex Lindl. 1833), called Hoang Kiem Lan in Vietnamese, is one of the orchids favorite to many orchidists. This is a native species of Vietnamese orchid belonging to the Orchidaceae family, *Cymbidium* genus. This genus consists of approximately 50 varieties in the world. There are 31 varieties in Vietnam. The genus of *Cymbidium* grows on trees, rocky cliffs, and in limestone soil. This genus has long narrow leaves, cone-shaped pseudobulbs that are encased in the leaf bases. The peduncle of the flower is usually long bearing many flowers [4]. Finlayson's Cymbidium in Vietnam is famous for its beautiful yellow flowers, long-lasting blossoms, fragrant, well-planted, pest resistant, and diligent flowering. In addition, Finlayson's Cymbidium symbolizes strength, power, and noble, so it is believed that *Cymbidium* growing is very good in terms of feng shui.

There is a growing demand for orchids, but natural exploitation and traditional propagation with the extraction method are insufficient to provide plants. Therefore, the application of seed orchid propagation with the initial seeds sown in a nutritious medium has brought high efficiency.

Corresponding: phanthiphuongnhi@huaf.edu.vn
Submitted: August 11, 2017; Revised: August 28, 2017; Accepted: October 24, 2017

There have been many studies on *in vitro* propagation of orchid [3], [5], [7], [9], [10]. Research on tissue culture of native orchids is focused on some major species such as *Rhynchostylis, Dendrobium anosmum, Rhynchostylis gigantea*, etc. To enrich the propagation material and orchid species, we conduct the research on the propagation of Finlayson's Cymbidium. Our goal is to find the optimal medium for shoot multiplication and complete plant formation for this species by tissue culture.

2 Material and method

2.1 Material

Fruit of Finlayson's Cymbidium was collected in Thua Thien Hue province, about 5–6 months old. The peel was green-yellow and undamaged.

2.2 Method

Sterilized sample: The orchid fruit was gently shaken in a diluted soap solution for 15 minutes. Then, the surface of fruit was brushed and rinsed slightly under running tap water. The cleaned fruit was put in a sterilized flask to sterilize with 70 % alcohol for 2 minutes. The orchid fruit surface was sterilized with a mixture of Javel solution and distilled water at 1:1 ratio for 15 minutes and rinsed 3 times with sterilized distilled water, every 10 minutes. The fruit was divided into two parts along the vertical line of fruit by a scalpel. The orchid seeds were transplanted into the sterilized flask containing various nutrient media.

Experimental design: Each experiment formulation was repeated three times in a completely randomized design with 3–5 samples/run. The experiments were evaluated after 4 weeks and 6 weeks of culture. Experimental formulas included varying concentrations of growth stimulators (6-benzyladenine–BA, 1-naphthaleneacetic acid–NAA), and coconut water (CW). The basal medium MS + 20 g/L saccharose + 6 g/L agar + 1 g/L activated charcoal (AC) was used as a control. MS is a notation for Murashige–Skoog mixture [1].

Conditions of culture: The experiments were carried out in the tissue culture room of the Department of Genetics and Breeding Plant, Faculty of Agronomy, University of Agriculture and Forestry, Hue University. Fluorescent light was used in the laboratory. Illumination time and intensity were 12 h/day and 2000 lux, respectively. The culture room temperature was 25 ± 2 °C with 50–70 % humidity. pH of culture medium was 5.6.

Evaluation indicators: Shoot multiplication = Total number of newly formed shoots /Total number of initial shoots. Number of leaves and of roots were counted from new leaves and roots.

The data were assessed with the analysis of variance by using the SSPS ver. 20 software, and the mean values were compared using Duncan's test ($\alpha = 0.05$).

3 Results and discussion

Effect of coconut water and 6-benzyladenine on protocorm regeneration

The orchid seed germination took place after 2 weeks to 4 weeks of culture depending on species. The Murashige–Skoog mixture is one of the most popular nutrient media for tissue culture [2]. Therefore, the MS medium supplemented with CW and BA was used to increase the efficiency of protocorm regeneration.

Table 1. Effect of coconut water and 6-benzyladenine on protocorm regeneration

No.	Medium	Protocorm morphology
1	MS	–
2	MS + CW 10 %	–
3	MS + CW 20 %	+
4	MS + 1 mg/L BA	+++
5	MS + CW 10 % + 1 mg/L BA	++++
6	MS + CW 20 % + 1 mg/L BA	++

Note: – means less protocorm, brown-yellow; + means small protocorm, yellow; ++ means medium grown protocorm, yellow; +++ means good grown protocorm, light green; ++++ means well grown protocorm, relatively uniform in size, green.

The germination of orchid seed in the different media was different (Table 1). In the control and the medium MS + 10 % CW, the seed germination rate was very small; the protocorm grew slowly. Meanwhile, in the medium MS + 10 % CW + 1 mg/L BA, the seed germination was good, uniform in size and color (green); the seeds germinated quickly (after 2 weeks). In other media, seeds also germinated, but the germination was not satisfactory nor as effective as the previous medium. Therefore, in this stage, we chose this medium as the starting material for the propagation of Finlayson's Cymbidium.

Effect of 6-benzyladenine on shoot multiplication

After the bud protocorm formation, the shoots were used as materials in the multiplication stage under the influence of growth stimulators BA and NAA.

After 2 weeks of culture on the MS medium supplemented with 2 mg/L BA (medium 5), the shoot multiplication factor was the highest at 1.87 with big, uniform shoots. The shoot appeared during 5–7 days, which was a relatively short time. The other media did not support the shoot multiplication much, and all the media did not show a significant difference at α = 0.05. After 4 weeks of culture on the same medium, the shoot multiplication factor was also the highest at 3.13 and was statistically significant at α = 0.05 compared with other media. However, when the BA concentration increased to 2.5 mg/L, the shoot multiplication factor decreased to 2.2. This revealed that high BA concentration negatively affects the shoot quality. This result is consistent with that of Phi Thi Cam Mien. In her *in vitro* propagation of

Anoectochilus setaceus Blume, she reported that very high or very low BA concentration gave small, weak and light-green shoots [6].

Table 2. Effect of 6-benzyladenine on shoot multiplication

No.	BA conc. (mg/L)	After 2-week culture		After 4-week culture	
		Shoot number	Shoot multiplication (times)**	Shoot number	Shoot multiplication (times)**
1	0.0	18	1.20^{a}	21	1.40^{d}
2	0.5	19	1.26^{a}	24	1.60^{cd}
3	1.0	23	1.53^{a}	28	1.87^{bcd}
4	1.5	22	1.47^{a}	30	2.00^{bc}
5	2.0	28	1.87^{a}	47	3.13^{a}
6	2.5	22	1.47^{a}	33	2.20^{b}

*** Values in the same column with the same letter indicate no significant difference ($p \leq 0.05$) according to Duncan's test.*

Effect of 6-benzyladenine and 1-naphthaleneacetic acid on shoot multiplication

The ratio of auxin/cytokinin is important for morphogenesis in the cultured systems [2]. Thus, combining BA and NAA at a certain ratio will stimulate or inhibit shoots or roots formation. In order to get good shoot quality, the combinations of BA and NAA at different concentrations were tested to find the optimal medium. The results after 2 weeks and 4 weeks of culture are illustrated in Table 3.

Table 3. Effect of 6-benzyladenine and 1-naphthaleneacetic acid on shoot multiplication

No.	BA conc. (mg/L)	NAA conc. (mg/L)	After 2 weeks culture		After 4 weeks culture	
			Shoot number	Shoot multiplication (times)**	Shoot number	Shoot multiplication (times)**
1	0.0	0.0	18	1.20^{c}	28	1.87^{c}
2	0.5	0.5	20	1.33^{bc}	28	1.87^{c}
3	0.5	1.0	23	1.53^{ab}	33	2.20^{b}
4	1.0	0.5	27	1.80^{b}	38	2.53^{a}
5	1.0	1.0	23	1.53^{ab}	31	2.07^{c}

*** Values in the same column with the same letter indicate no significant difference ($p \leq 0.05$) according to Duncan's test.*

After 2 weeks of culture, the medium MS + 1 mg/L BA + 0.5 mg/L NAA had the highest shoot multiplication factor at 1.8 and statistically significant at α = 0.05 compared with the control. However, there was no statistically significant difference compared to other media. After 4 weeks of culture, the same medium gave the highest shoot multiplication factor at 2.53. This time, the difference was statistically significant compared with other formulas at α = 0.05.

If we compare the results shown in Table 2 and Table 3, we can see that despite the fact that the combination of two stimulators can boost the shoot reproduction. This effect is not as high as that at the medium MS + 20 g/L saccharose + 6 g/L agar + 1 g/L AC supplemented with 2 mg/L BA with the shoot multiplication factor at 3.13 as opposed to 2.53 for the case of two stimulators.

Effect of 1-naphthaleneacetic acid on the root number after culture

In the previous experiments, when the leaves appeared, the shoots grew and developed slowly with a poor quality. Therefore, in the next experiment, NAA with different concentrations was used to improve the root, leave and height parameters of Finlayson's Cymbidium. The results are shown in Tables 4, 5 and 6.

After 2 weeks of culture, the number of roots/plant in the media was not different. After 4 weeks of culture, the medium MS + 1mg/L NAA gave the highest number of roots/plant at 2.80. However, there was no statistical difference among the media 3, 4, 5, 6 and 7 at $\alpha = 0.05$. After 6 weeks of culture, the above media also gave the highest number of roots/plant, but this time the difference is not statistically significant either (Table 4).

Table 4. Effect of 1-naphthaleneacetic acid on the root number after culture

No.	NAA conc. (mg/L)	Number of root per plant (root)**		
		2 weeks	4 weeks	6 weeks
1	0.0	0.60^{a}	1.40^{c}	1.60^{c}
2	0.1	0.60^{a}	1.80^{bc}	2.00^{bc}
3	0.3	0.40^{a}	2.40^{ab}	2.40^{ab}
4	0.5	0.60^{a}	2.60^{a}	2.60^{ab}
5	0.7	0.80^{a}	2.40^{ab}	2.60^{ab}
6	1.0	1.00^{a}	2.80^{a}	3.00^{a}
7	1.5	0.60^{a}	2.40^{ab}	2.40^{ab}

*** Values in the same column with the same letter indicate no significant difference ($p \leq 0.05$) according to Duncan's test.*

Table 5 shows the effect of 1-naphthaleneacetic acid on the leaf number. It can be seen that the plants had about one to two leaves after two weeks, and this number increased after 4 weeks and 6 weeks of culture. In fact, after four weeks, the average number of leaves per plant in the medium MS + 0.7 mg/L NAA and medium MS + 1.5 mg/L NAA was the same at 2.6. However, there was no statistically significant difference among medium 5, 6, and 7. After six weeks, leaf numbers ranged from 2.2 to 3.8 leaves per plant. Similar to the results after four weeks, there was also no statistically significant difference among medium 5, 6, and 7.

The effect of 1-naphthaleneacetic acid on the plant height is demonstrated in Table 6. The plant height in the media was not high, ranging from 1.18 cm to 1.72 cm after 2 weeks of culture. After 4 weeks of culture, the plant height increased and reached the highest value with medium 5 (3.32 cm). After the 6-week culture, the plant was 5.34 cm high with the medium

containing 0.7 mg/L NAA. This value is statistically significant at α = 0.05 compared with the heights obtained from the remaining media. Thus, this was considered as the optimal medium for Finlayson's Cymbidium culture. The plants can grow in the green house after the *in vitro* stage. Tran Thi Hong Thuy et al. also suggested that the *in vitro* orchid plant having an average height of 5 cm, 4 leaves and 3 roots could be transplanted in the green house [8].

Table 5. Effect of 1-naphthaleneacetic acid on the leaf number after culture

No.	NAA conc. (mg/L)	Number of leaf per plant**		
		2 weeks	4 weeks	6 weeks
1	0.0	1.20^{a}	1.60^{c}	2.20^{c}
2	0.1	1.40^{a}	1.60^{c}	2.20^{c}
3	0.3	1.60^{a}	2.00^{abc}	2.80^{bc}
4	0.5	1.40^{a}	1.80^{bc}	3.00^{b}
5	0.7	1.80^{a}	2.60^{a}	3.80^{a}
6	1.0	1.40^{a}	2.40^{ab}	3.20^{ab}
7	1.5	1.60^{a}	2.60^{a}	3.40^{ab}

*** Values in the same column with the same letter indicate no significant difference ($p \leq 0.05$) according to Duncan's test.*

Table 6. Effect of 1-naphthaleneacetic acid on the plant height after culture

No.	NAA conc. (mg/L)	Plant height (cm)**		
		2 weeks	4 weeks	6 weeks
1	0.0	1.18^{d}	2.68^{d}	3.84^{e}
2	0.1	1.26^{d}	2.88^{c}	4.04^{d}
3	0.3	1.54^{b}	3.12^{b}	4.10^{d}
4	0.5	1.38^{c}	3.20^{ab}	5.08^{b}
5	0.7	1.72^{a}	3.32^{a}	5.34^{a}
6	1.0	1.58^{b}	3.18^{ab}	5.10^{b}
7	1.5	1.52^{b}	3.08^{b}	4.62^{c}

*** Values in the same column with the same letter indicate no significant difference ($p \leq 0.05$) according to Duncan's test.*

4 Conclusion

The combination of coconut water and 1-naphthaleneacetic acid stimulated the growth of the protocorm bubs rather than using coconut water or BA separately. The MS medium supplemented with 20 g/L saccharose, 6 g/L agar, 1g/L activated charcoal, 10 % coconut water and 1 mg/L BA was the optimal medium for protocorm shooting of Finlayson's Cymbidium.

The MS medium supplemented with 1g/L activated charcoal, 20 g/L saccharose, 6 g/L agar and 2 mg/L BA was the most suitable for shoot propagation stage; shoot multiplication factor was 3.13 times after 6 weeks of culture.

For the rooting and complete plant formation stage, the MS medium supplemented with 20g/L saccharose, 6 g/L agar, 1 g/L activated charcoal and 0.7 mg/L NAA was the optimal medium in this experiment. After 6 weeks of culture, the number of roots/plant reached 2.6, and the number of leaves/plant was 3.8; the plant height was 5.34 cm.

References

1. Murashige, T. and Skoog F. (1962), A revised medium for rapid growth & bioassay with tobacco tissue cultures. *Physiol. Plant*, 15: 473–497.
2. Nguyen Hoang Loc (2011), *Plant cell and tissue*, Hue University Publish house (in Vietnamese).
3. Nguyen Văn Song (2011), In vitro propagation Dendrobium chrysotoxum – a forest orchid that is extinction, *Hue University Journal of Science*, 64: 127–136 (in Vietnamese).
4. Nguyen Thien Tich, Đoan Thi Hoa, Tran Si Dung, Huynh Thi Ngoc Nhan (2003), *Orchid technical cultivation*, Agriculture Publish house (in Vietnamese).
5. Park, S. Y., Murthy, H. N. and Paek, K. Y. (2002), Rapid propagation of Phalaenopsis from floral stalk – derived leaves, *In Vitro Cell, Dev. Biol. Plant* 38: 168–172.
6. Phi Thi Cam Mien (2012), Research on in vitro propagation of orchid *Anoectochilus setaceus* Blume to conserve precious medicinal resources, Master Thesis, Hanoi University of Science (in Vietnamese).
7. Tawaro S., Suraninpong P. and Chanprame S. (2008), Germination and Regeneration of Cymbidium findlaysonianum Lindl.on a Medium Supplemented with Some Organic Sources. *Walailak J. Sci. & Tech*. 5 (2): 125–135.
8. Tran Thi Hong Thuy, Do Thi Gam, Nguyen Khac Hung, Pham Bich Ngoc, Chu Hoang Ha (2015), *In vitro* micropropagation of an endangered medicinal orchid (*Anoectochilus setaceus* Blume) through protocorm-like bodies. *J. Bio*., 37 (1): 76–83 (in Vietnamese).
9. Vo Thi Bach Mai (1996), Clonal propagation of some orchids (Phalaenopsis sp.). Ph.D. Desertation of Biological Science, Ho Chi Minh, 6–87 (in Vietnamese).
10. Vu Ngoc Lan and Nguyen Thi Ly Anh (2013), *In vitro* micropropagation of Wild Orchid *Dendrobium nobile* Lindl. *J. Sci. & Devel*. 11 (7): 917–925 (in Vietnamese).