Effect of Plant Growth Regulators on Clonal Production through Basal Stem Explant Cultures of a *Phalaenopsis* Hybrid

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ABSTRACT

This study was conducted to develop the clonal propagation technique through *in vitro* culture using basal stem explants in *Phalaenopsis* hybrid grown *in vitro*. The highest frequency of protocorm-like body (PLB) formation was obtained when basal stem explants were cultured on VW medium containing 30g/L sucrose, 500 mg/L activated charcoal, 150 ml/L coconut water, 1 mg/L NAA, 5 mg/L 2iP and 2.5g/L gelrite. PLBs transferred to Hyponex medium were regenerated to plantlets. Plantlets transferred to plastic pots containing spagnum moss were developed and successfully acclimatized under greenhouse. The flower was bloomingly opened in plants regenerated from basal stem explants. The flower was not different from both mother plant and plant induced through clonal propagation of *Phalaenopsis* hybrid.

Key words **:** flowering, PLB, regeneration, VW medium

INTRODUCTION

Recently, orchid growing is more than just an industry, it is an international business. It is widely recognized that potted *Phalaenopsis* production has increased tremendously in the last few years. The popularity of *Phalaenopsis* naturally led to the creation of many artificial hybrids. *Phalaenopsis* are often also called, monopodial plants. The genus has approximately 50 species. The range of this genus is from India, through Southeast Asia, North to the Philippines, and South to Northern Australia. The species are epiphytic or lithophytic and inhabit areas from sea level to 1,000 feet. Currently, the production of pot plants and cut flowers of *Phalaenopsis* has increased greatly throughout the world.

In *Phalaenopsis*, tissue culture propagation produced too much genetic variation. In some instances, over 50% of the propagated plants produced flowers that were significantly different from the mother plant. Therefore, mass-market *Phalaenopsis* were propagated not by vegetatively, but by seed. Recently, most of the commercially important orchids cultivars such as *Cattleya, Dendrobium* and *Cymbidium* propagated vegetatively through tissue culture have been predominantly used for production. However, most of the seeds are heterozygous resulting in the high variability in qualitative and quantitative character of

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Phalaenopsis. Although there have been several reports on successful micropropagation of *Phalaenopsis* using different explant sources (Homma and Asahira, 1985; Tokuhara and Mii, 1998), clonal micropropagation is still not popular in this orchid because of the difficulties such as low multiplication rate and occurrence of somaclonal variations in applying the methods to large scale production of plantlets. For commercial micropropagation of plants, the occurrence of somaclonal variation is one of the most serious problems. In *Phalaenopsis*, a low frequency of somaclonal variation was reported previously by Tokuhara and Mii (1998) using a relatively large number of plants. However, it is necessary to use a large number of plants for appropriate evaluation of frequency of variations which might occur in commercial scale micropropagation. Moreover, PLBs obtained through some of these methods did not proliferate readily and their viability was low due to phenolic compounds derived from tissue. Micropropagation capability of *Phalaenopsis* vary according to cultivar. The present study was conducted to develop the clonal propagation technique through *in vitro* culture by using basal stem explants of *Phalaenopsis* hybrid grown *in vitro*.

MATERIALS AND METHODS

Plant materials

Protocorm-like body derived from the basal stem explants in *Phalaenopsis* hybrid (*Phal. aphrodite* × 'Malibu') was propagated by subculturing in VW solid medium (VW, 1949). The pH of the medium was adjusted to 5.3 before autoclaving at 121℃ for 15 minutes. The explants in culture tubes were incubated at 25±1℃ on continuous light (20μmol · m^{-2} · s^{-1}) provided by white fluorescence lamps.

Preparation of basal stem explants

The basal stem explants (average length 3.0 mm × 5.0 mm) were aseptically excised.

Culture medium for basal stem explants

The culture medium was the basal VW solid medium (containing 3% sucrose, 500 mg/L activated charcoal, 150 ml/L coconut water (CW) and 0.25% gelrite) and supplemented with 2 levels of NAA (0.1 and 1 mg/L), 3 levels of BA (0.1, 1.0, and 5.0 mg/L), and 3 levels of 2iP (0.1, 1.0, and 5.0 mg/L).

Effect of plant growth regulators on growth and development of PLB from basal stem explants

A completely randomized design was used, and each treatment was 25 explants. The culture tubes were incubated at 25±1℃ on continuous light (20μmol · m^{-2} · s^{-1}) provided by white fluorescence lamps. After 8 weeks of culture, the cultured explants in all treatments were scored by the percentage of explants forming PLBs.

Flowering through PLB

For plantlets regenerated from PLBs in 10 mg were excised and cultured on VW medium supplemented with 3% sucrose, 500 mg/L activated charcoal, 150 mg/L CW, 1.0 mg/L NAA, 5.0 mg/L 2iP with 0.25% gelrite. Each PLB was cultured with 500 mL mayonaise bottles containing 80 mL medium. Cultures were kept at 25±1℃ for 16 hours photoperiods with 30 μmol · m^{-2} · s^{-1} Photosynthetic Photon Flux (PPF). The plantlets from PLB transferred to plastic pots (ø 80 mm × h75 mm) containing New Zealand spagnum moss in greenhouse. The *Phalaenopsis* hybrid have been fertilized with Peters (20N-20P-20K) to provide the 1,000-fold diluted solution. The flower was bloomingly opened in healthy plants regenerated.

RESULTS AND DISCUSSION

Table 1. Effect of plant growth regulators on protocorm-like body formation derived from cultured basal stem explants in *Phalaenopsis* hybrid for 8 weeks

Plant growth regulators (mg/L)			No. of basal stem explants cultured	No. of PLB	PLB (%)
NAA	BA	2iP			
0.1	0.5		25	1	4.0
0.1	1.0		25	0	0
0.1	5.0		25	5	20.0
0.1		0.5	25	2	8.0
0.1		1.0	25	2	8.0
0.1		5.0	25	3	12.0
1.0	0.5		25	1	4.0
1.0	1.0		25	3	12.0
1.0	5.0		25	2	8.0
1.0		0.5	25	1	4.0
1.0		1.0	25	0	0
1.0		5.0	25	6	24.0

The present study was carried out to establish the convenient methods for clonal propogation through basal stem explants of *Phalaenopsis* hybrid *in vitro*. It was found that when basal stem explants were cultured on various different levels of concentrations of modified VW medium, the highest number of PLB multiplication was obtained from the VW medium including 1.0 mg/L NAA and 5 mg/L 2iP at 20 μmol · m^{-2} · s^{-1} in PPF and 25±1℃ for 8 weeks. PLBs transferred to VW medium were developed to plantlets. Plantlets transferred to plastic pots containing spagnum moss were developed and successfully acclimatized under greenhouse. No differences were found from both mother plant and plant induced through clonal propagation in flower of *Phalaenopsis* hybrid.

Effect of modified VW medium and plant growth regulators on plantlet regeneration through protocorm-like body formation derived from cultured basal stem explants in *Phalaenopsis* hybrid was observed in the present study was shown in Table 1. The frequency of

Fig. 1. Plant regeneration of clonal propagation through basal stem explants in *Phalaenopsis* hybrid *in vitro*. A: PLBs induced from basal stem explants of Phalaenopsis after 60 days of culture; B: Plantlets derived from PLB; C: Seedling growing in a plastic pot in the greenhouse.

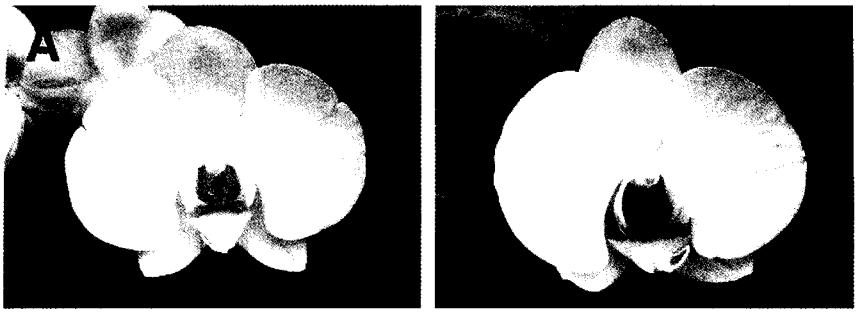

Fig. 2. Comparison between flowers of mother plant (A) and plant regenerated (B) of clonal propagation through basal stem explants in *Phalaenopsis* hybrid.

PLB formation per explants on modified VW medium ranged from 0 to 24%. The highest frequency(24%) of PLB formation was produced when basal stem explants were cultured on VW medium containing 30 g/L sucrose, 500 mg/L activated charcoal, 150 ml/L CW, 1 mg/L NAA, 5 mg/L 2iP and 2.5 g/L gelrite. However, the growth of PLBs in the cultures was not uniform. The VW gelrite medium was found to be the most suitable for formation and growth of PLB when compared to other media used in the previous study (Jo et al., 2002).

Shoot regeneration and basal stem explants derived PLB, and its subsequent growth was promoted by culturing on Hyponex medium, and were reprolifirated on the PLB medium containing 1 mg/L NAA and 1 mg/L 2iP. The PLB were formed on the surface of adaxial side in *Phalaenopsis* (Tanaka *et al.*, 1975). Depending on the hormone combination, PLB formation was affected, but there will be always a risk of somaclonal variations when plant growth regulators were used. There was a synergistic effect of 1 mg/L NAA and 5 mg/L 2iP on PLB formation (Table 1). The PLB were green in color and propagated well in subsequent culture. It was assumed that VW medium was also a proper medium for PLB induction from basal stem explants.

Concentration of plant growth regulator is one of the factors PLB induction. These results suggest that VW medium containing 1.0 mg/L NAA and 5.0 mg/L 2iP with 2.5 g/L gelrite was the best optimal medium for PLB propagation. In previous studies, high BA concentration(10 mg/L) was used to induce PLBs on emerging leaves of seedlings and mature plant (Tanaka *et al.*, 1975; Tanaka and Sakanish, 1985; Myint *et al.*, 2001).

In the present study, however, PLBs were induced from basal stem explants cultured on all of the VW medium containing low 3 levels of BA (0.1, 1.0, and 5.0 mg/L) and low 3 levels of 2iP (0.1, 1.0, and 5.0 mg/L). PLBs transferred to VW medium were developed to plantlets. Plantlets transferred to plastic pots containing spagnum moss were developed and successfully flowered under greenhouse (Fig. 1A-2B). PLBs induced from basal stem explants of *Phalaenopsis* hybrid after 8 weeks of initial culture (Fig. 1A). In the present study, plantlets derived from PLBs were observed after transfer onto the VW medium containing 30g/L sucrose, 500 mg/L activated charcoal, 150 ml/L CW, 1 mg/L NAA, 5.0 mg/L 2iP with 2.5 g/L gelrite after three months of culture (Fig. 1B). Young *Phalaenopsis* hybrid was developed and successfully grown under greenhouse (Fig. 1C). *Phalaenopsis* hybrids were successfully flowered from mother plant (Fig. 2A) and plant regenerated of clonal propagation through basal stem explants under greenhouse (Fig. 2B). In this study, we investigated the effects of plant growth regulators and culture media on PLB formation and plant regeneration from basal stem

explants. The plant growth regulators, cytokinins and auxins, affect differentiation of adventitious shoots, somatic embryos, adventitious roots, and callus formation in vitro (Fujii *et al.*, 1999). In this study, PLBs were produced directly in a different media with different plant growth regulators from basal stem explants of *Phalaenopsis* hybrid. About 30% of the *Phalaenopsis* hybrid through PLBs were developed and successfully flowered in a plastic pot containing spagnum moss under greenhouse whereas the remaining plants were unhealthy. The most important of clonal propagation was highly uniform in all characters of clonal progeny.

Although the flowering involved in the plant regeneration and formation of these PLB are still unclear in *Phalaenopsis* hybrid. Further study should be required to understand various factors associated with the optimization for clonal propagation, a uniform growth and flowering.

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