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Clonal Propagation through Leaf Sheath Culture of Phalaenopsis

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ABSTRACT

This study was conducted to develop the clonal propagation technique through in vitro culture using by leaf sheath explants of Phalaenopsis grown in vitro. The highest frequency of protocorm-like body (PLB) formation was obtained when explants of leaf sheath were cultured on VW medium containing 30g/L sucrose, 500 mg/L activated charcoal, 150 ml/L coconut water, 1 mg/L NAA, 1 mg/L 2ip and 2.5 g/L gelrite. The PLB formation rate of VW medium was highest followed by modified Hyponex medium, and lowest in MS medium. Plantlets induced from PLBs transferred to plastic pots including spagnum moss were well developed.

Key Words : orchid, PLB, regeneration, VW medium

INTRODUCTION

Phalaenopsis are often also called, monopodial plants. The genus has some 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 most of the commercially important orchids such as Cattleya, Dendrobium and Cymbidium, cultivars propagated vagetatively through tissue culture are now predominantly used for production. However, the most of the seeds are heterozygous resulting in the high variability in qualitative and quantitative character of Phalaenosis. 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

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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 using by leaf sheath explants of Phalaenopsis grown in vitro.

MATERIALS AND METHODS

Plant materials

Protocorm-like body derived from the leaf sheath explants in white yellow lip strain of Phalaenopsis were propagated in modified VW solid medium (Vacin and Went, 1949), modified Hyponex medium, MS medium(Murashige and Skoog, 1962). The pH of the medium was adjusted to 5.3 before autoclaving at 121 ℃ for 15 minutes. The explants in culture test tubes(ø 25 mm × h150 mm) were incubated at 25±1℃ and 10 μmol · m^{-2} · s^{-1} photosynthetic photon flux(PPF) for 2 weeks and then transferred to 30 μmol · m^{-2} · s^{-1} PPF with a 16-h light provided by white fluorescence lamps.

Preparation of leaf sheath explants

The leaf sheath explants (average length 3.0 mm × 5.3 mm) was aseptically excised.

Culture medium for leaf sheath explants

The culture medium was the VW medium (containing 3% sucrose, 500 mg/L activated charcoal, 150 ml/L coconut water(CW) and 2.5 g/L gelrite), modified Hyponex medium(including 3% sucrose, 500 mg/L activated charcoal, 3.5 g/L Hyponex ($N:P_2O_5:K_2O$), 4 g/L peptone, 100 mg/L myo-inosytol, 1 mg/L nicotinic acid, 1 mg/L thiamine · HCl, 10 mg/L adenine, and 2.5 g/L gelrite), MS medium (containing 3% sucrose, 500 mg/L activated charcoal, and 2.5 g/L gelrite) and supplemented with plant growth regulator(PGR) 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 media and additives on growth and development of PLB from leaf sheath explants

A completely randomized design was used, and each treatment was with 37 explants (VW), 20 explants (modified Hyponex), and 20 explants (MS), respectively. The test tube were cultured on modified VW, modified Hyponex, and MS medium, at 25±1℃ with a 16-h light 20 μmol · m^{-2} · s^{-1} PPF and then transferred to 30 μmol · m^{-2} · s^{-1} PPF. After 8 weeks of culture, the cultured explants in all treatments were scored for the percentage of explants forming PLBs.

Phalaenopsis plant regeneration and acclimataization

For plantlet regeneration from PLBs in 10 mg which were excised and cultured on VW medium supplemented with 3% sucrose, 500 mg/L activated charcoal, 150 mg/L CW, 0.1 mg/L NAA, 5.0 mg/L 2ip with 2.5 g/L 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} PPF. The plantlets from PLB transferred to plastic pots (ø 80 mm × h75 mm) containing New Zealand spagnum moss in greenhouse. The Phalaenopsis have been fertilized with Peters(20N-20P-20K) to provide the 1,000-fold diluted solution.

RESULTS AND DISCUSSION

Effect of media and plant growth regulators on plantlet regeneration through protocorm-like body formation derived from cultured leaf sheath explants in Phalaenopsis observed in the present study was shown in Table 1. The frequency of PLB formation per explants on modified VW medium ranged from 3% to 19% while PLB formation ranged from 5% to 10% on modified Hyponex and MS medium. The highest frequency(19%) of PLB formation was produced when

Table 1. Effect of media and plant growth regulators on protocorm-like body formation derived from cultured leaf sheath explants in Phalaenopsis for 8 weeks.

Medium	PGR (mg/L)			No. of explants	No. of PLBs	PLB (%)
	NAA	BA	2ip			
VW	0.1	0.5		37	5	14
	0.1	1.0		37	3	8
	0.1	5.0		37	3	8
	0.1		0.5	37	1	3
	0.1		1.0	37	6	16
	0.1		5.0	37	6	16
	1.0	0.5		37	1	3
	1.0	1.0		37	4	11
	1.0	5.0		37	3	8
	1.0		0.5	37	4	11
	1.0		1.0	37	7	19
	1.0		5.0	37	4	11
MS	0.1	0.5		20	0	0
	0.1	1.0		20	0	0
	0.1	5.0		20	0	0
	0.1		0.5	20	0	0
	0.1		1.0	20	0	0
	0.1		5.0	20	0	0
	1.0	0.5		20	1	5
	1.0	1.0		20	0	0
	1.0	5.0		20	0	0
	1.0		0.5	20	0	0
	1.0		1.0	20	1	5
	1.0		5.0	20	0	0
Hyponex	0.1	0.5		20	1	5
	0.1	1.0		20	0	0
	0.1	5.0		20	0	0
	0.1		0.5	20	2	10
	0.1		1.0	20	0	0
	0.1		5.0	20	2	10
	1.0	0.5		20	0	0
	1.0	1.0		20	0	0
	1.0	5.0		20	0	0
	1.0		0.5	20	1	5
	1.0		1.0	20	0	0
	1.0		5.0	20	0	0

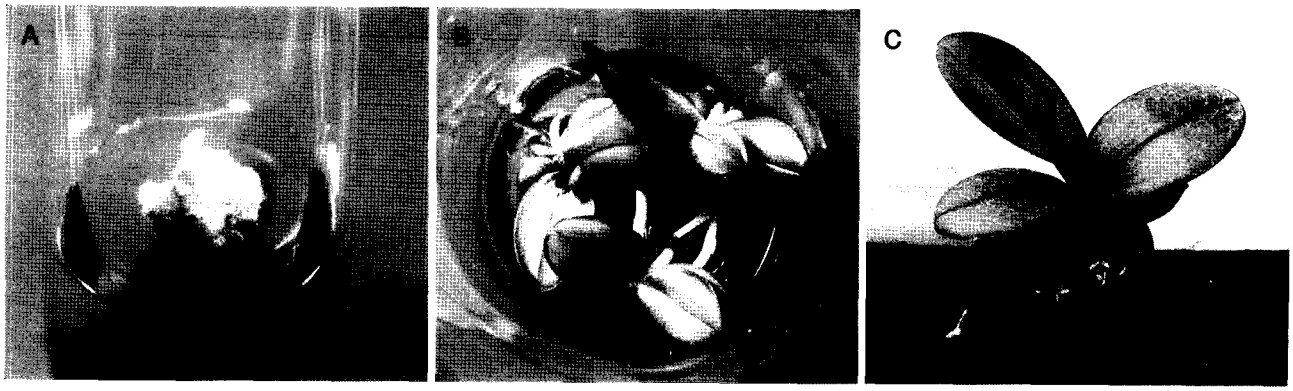

Fig. 1. Plant regeneration of clonal propagation through leaf sheath explants of Phalaenopsis in vitro. A: PLBs induced from adaxial surface of leaf sheath of Phalaenopsis on VW medium supplemented with 0.1 mg/L NAA and 1.0 mg/L 2ip; B:Plantlets derived from PLBs. Three months after transferring to the medium contained VW medium supplemented with 30g/L sucrose, 500 mg/L activated charcoal, 150 ml/L CW, 0.1 mg/L NAA, and 5.0 mg/L 2ip with 2.5 g/L gelrite; C:Regenerated plant potted in plastic pots (Ø 80 mm × h75 mm) containing spagnum moss in greenhouse.

leaf sheath explants were cultured on VW medium containing 30g/L sucrose, 500 mg/L activated charcoal, 150 ml/L CW, 1 mg/L NAA, 1 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 the most suitable for formation and growth of PLB when compared to other media used in this study.

Shoot regeneration and on leaf sheath explants derived PLB its subsequent growth was promoted by culturing on Hyponex medium, and reprolifiration of 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 are used. There was a synergistic effect of 1 mg/L NAA and 1 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 leaf sheath explants.

Concentration of PGR is one of the factors PLB induction. These results suggest that VW medium containing 1.0 mg/L NAA and 1.0 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 leaf sheath 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 acclimatized under greenhouse (Fig. 1). PLBs induced from leaf sheath of Phalaenopsis after 8 weeks days of initial culture(Fig. 1A). Our present study accords well with report(Tanaka *et al.*, 1975) that PLBs were formed on the surface of adaxial side in Vanda and Phalaenopsis. In the present study, plantlets derived from PLBs was observed after transfer onto the VW medium containing 30g/L sucrose, 500 mg/L activated charcoal, 150 ml/L CW, 0.1 mg/L NAA, 5.0 mg/L 2ip with 2.5 g/L gelrite after three months of

culture (Fig. 1B). Young Phalaenopsis were developed and successfully grown under greenhouse (Fig. 1C). In this study, we investigated the effects of plant growth regulators and culture media on PLB formation and plant regeneration from leaf sheath 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 PGRs from leaf sheath explants of Phalaenopsis. Plantlets of PLBs were developed and successfully acclimatized in a plastic pot containing spagnum moss under greenhouse.

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

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