IN VITRO CLONAL PROPAGATION OF PHALAENOPSIS THROUGH YOUNG LEAF

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Abstract

Phalaenopsis is an elegant orchid genus containing 62 species and mainly used for commercial production as cut flower and potted plant. Phalaenopsis orchid brings high profit in flower markets around the world. In Myanmar, Phalaenopsis is one of the famous and expensive genera among the orchids. Tissue culture became the important method for propagation of Phalaenopsis because of its growth habit of being difficult to propagate vegetatively. Explant types, plant growth regulators and culture system are mainly attributed to the success in the tissue culture propagation technique. In this experiment, in vitro leaf explants were studied for their PLBs induction potential in response to different kinds of plant growth regulator (BA, TDZ and NAA), different concentrations (2, 4 BA, 1, 2 TDZ and 0.5 NAA) and combinations of PGRs (BA + NAA, TDZ + NAA) and two culture systems (solid and cotton support liquid medium). Among the PGRs, highest PLBs induction was observed on ½ MS medium containing 2 mg.L-1BA. Interaction effect was observed between plant growth regulators and culture system. The effects of culture systems were not statistically different from each other. The PLBs obtained from TDZ supplemented media gave better result in solid culture than cotton culture. In contract, PLBs obtained from BA supplemented media showed better result in cotton culture than solid culture. Therefore, the use of PGRs should be selected depending on the culture system.

Key words: Phalaenopsis amabilis, PLBs induction, Culture systems, PGR

Introduction

Phalaenopsis amabilis is one of the popular orchid flowers and valued due to its different shape of beautiful flower, color and vase life. These are mainly cultivated for attractive cut flowers and potted plant. Phalaenopsis is a monopodial epiphytic orchid. In Myanmar, Phalaenopsis is one of the most famous and expensive genus that is mainly used as cut flower for various ceremonies like wedding and religious ceremonies, etc and potted plant for home decoration. It is mainly imported from Thailand and China. Phalaenopsis orchid cultivation and production became less in Myanmar due to the poor cultivation techniques. To improve the orchid market in Myanmar, better cultivation and production techniques are required.

Phalaenopsis is difficult to propagate vegetatively by conventional methods and many uniform and virus free plantlets cannot be obtained from this method within a short period. Therefore, tissue culture methods are the way for clonally mass propagation of commercial species. Many in vitro propagation protocols have been established for Phalaenopsis propagation using various plant parts such as flower stalk buds, stem node (Tokuhara and Mii 2001), entire shoots, shoot tip (Griesbach 2002), and leaf tissues (Park et al. 2002). Propagation by using various explants obtained different survival%, multiplication and number of plantlets. Among them, proliferation of protocorm-like bodies (PLBs) developed from leaf culture has been widely used for propagation of Phalaenopsis and that method cannot damage the mother plant. PLBs are assumed

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as somatic embryo which can be induced directly from various explants. Therefore PLB is efficient material in micropropagation and it can be rapidly proliferated on different culture system.

Plant growth regulators (PGRs) and culture systems are one of the major factors to get the successful propagation techniques in tissue culture. Plant growth regulators such as auxin, cytokinin, gibberellins and abscisic acids are used in tissue culture. Among them, auxin and cytokinin are mainly used for development of PLBs or callus, shoots and plantlets growth (Arditti and Ernst, 1993). However, PGRs type, concentration and combination of hormones vary according to the explant types, species and expected product. Therefore, suitable PGRs type, concentration and combination were tested to obtain the expected product in this study.

Another major factor is culture systems, which influence the growth of explants by upholding the nutrient. Culture system can be used as solid, semi-solid and liquid types in tissue culture. Different types of culture systems provide different effects for orchid regeneration. Based on this information, the objective of this experiment was to observe the effect of plant growth regulators (PGRs) and culture systems on PLBs induction from leaf explants of Phalaenopsis amabilis orchid.

Materials and Methods

In this experiment, young emerging leaves from in vitro plantlets were used as explants. Two kinds of culture systems; solid and cotton support liquid culture, and different PGRs, concentration and combination were tested for PLB induction in initial culture stage. Young leaves (0.5 – 2 cm) were excised into 1- 2 mm cross section and cultured on the ½ MS medium supplemented with different concentrations of BA (2, 4 mg.L-1), TDZ (1, 2 mg.L-1) individually or in combination with 0.5 mg.L-1 NAA. Sucrose 20 g.L-1 and 7 g.L-1 agar were added in this medium and adjusted at pH 5.7 before autoclaving for 15minutes. Three explants were cultured in each vessel. Cultures were incubated in dark for 2 weeks and then transferred to 16 hour photoperiod, 20-40 µmolm2s-1 and temperature 25 ± 1 °C. After 6 week culture, PLBs emerged and then they continued to develop. Survival percent, PLBs formation percent and number of PLBs per explant were collected within 6 to 10 weeks. Two factors factorial randomized complete block design with 4 replications was used for this experiment. Twelve explants were cultured in each treatment and the effect of culture systems and hormonal treatments on PLB induction were investigated respectively.

Data Analysis

Table 1. Effect of different PGRs and culture system on survival %, PLBs formation % and number of PLBs per explant in PLBs induction of leaf culture

Plant Growth Regulators (mg.L^{-1})	Survival %	PLBs formation %	Number of PLBs per explant
2.0 BA + 0.0 NAA	44.6 bcd	76.6 a	7.1 a
4.0 BA.+ 0.0 NAA	45.8 abcd	68.1 a	5.4 ab
2.0 BA.+ 0.5 NAA	41.6 cd	81.2 a	4.8 abc
4.0 BA + 0.5 NAA	34.4 d	65.0 ab	2.3 c
1.0 TDZ + 0.0 NAA	57.4 abc	37.4 b	2.6 c
2.0 TDZ + 0.0 NAA	49.0 abcd	58.4 ab	5.9 ab
1.0 TDZ + 0.5 NAA	61.4 a	57.9 ab	3.6 bc
2.0 TDZ + 0.5 NAA	59.3 ab	58.9 ab	2.3 c
$LSD_{0.05}$	16.5	28.9	2.6
Culture Systems (CS)			
Solid culture	51.5 a	61.7 a	4.6 a
Cotton culture	46.8 a	64.2 a	4.4 a
$LSD_{0.05}$	8.3	14.5	1.3
CV%	33.5	45.7	57.6
Pr>F			
PGRs	*	ns	**
CS	ns	ns	ns
PGRs * CS	*	ns	**

Means followed by the same letter in each column are not significantly different at 5% level. Data were collected from 6 weeks to 10 weeks after cultured.
ns: No significant ** Significant at 1% level * Sig-

The data were statistically analyzed by using SAS program and mean comparisons were performed using Least Significant Difference (LSD) at 5% level.

Results and Discussion

PLBs were induced from thin leaf explants after 6 weeks cultures on ½ MS medium containing various PGRs in two different culture systems. A significant variation was observed among the PGRs treatments in survival % and number of PLBs per explants but not observed in culture systems (Table 1). Among the PGRs treatments, maximum survival percentage 61.4 was obtained from the combination of 1 mg.L-1 TDZ and 0.5 mg.L-1 NAA. Although, Culture systems were not statistically different between each other, solid culture system gave better survival percent than cotton culture system. In this parameter, explants showed good survival in all TDZ treatments. Guo et al. (2011) reported that TDZ can activate the survival apparatus of plant tissue used for asexdual reproduction.

In PLBs formation percent, there was no significant difference among the PGRs treatments and culture systems. But maximum percentage 81.2 was observed from the combination of 2 mg.L-1 BA and 0.5 mg.L-1 NAA. Sinha and Jahan, 2011 and Sinha et al. 2010 found that BA-NAA combinations increased the rate of PLB induction and the optimum concentration of BA and NAA was 2.0 and 0.5 mg.L-1 in which 80% of PLBs formation was observed in Phalaenopsis amabilis (L.) BL. cv. 'Golden Horizon' and 75% PLBs formation was found in Phalaenopsis amabilis (L.) Bl. cv.Lovely. However, the rate of PLBs formation % and number of PLBs per explant vary depending on the species. It would be due to the effect of different cultivars that can vary in regeneration capacity. In this parameter, explants showed better PLBs formation % in all BA supplemented medium than TDZ supplemented medium.

In number of PLBs per explants, the results were highly significantly different among the PGRs treatment. Maximum 7.1 PLBs per explant was observed in media containing 2 mg.L-1 BA. Feng and Chen (2014) and Rittirat et al. (2014) studied the effect of TDZ and BA on the protocorms induction in two orchid species, P. cornu-cervi and P. aphrodite spp. Formosana. In their study, TDZ is more effective than BA in PLB induction but the current study was against with these results. It might be due to the possible effects of different genotypes (Myint et al. 2006). Balilashaki and Ghehsareh (2016) also concluded that BAP was more helpful to obtain maximum number of PLBs per explant than TDZ in Phalaenopsis amabilis var. 'Manila'.

Interaction effect was observed among the culture system and PGRs in survival % and number of PLBs per explant parameters. The effects of hormones depend on the culture system. In survival % and number of PLBs per explants parameters, TDZ was better than BA in solid culture (Figure 3) and, BA was better than TDZ in cotton culture (Figure 2). PLBs from leaf explants cultured on the BA supplemented cotton support liquid culture showed better appearance than PLBs on solid culture medium (Figure 1). PLBs obtained from TDZ supplemented cotton support liquid culture showed poor performance. (Figure 4) . It might be due to not only the effect of TDZ and BA but also the nutrient maintenance capacity of culture systems. TDZ is more active than BA. A solid culture has better nutrient maintenance capacity than a cotton culture does. Therefore, TDZ should be used in solid cultures and BA in cotton cultures. Thus, the solid culture will release TDZ to explants slowly and therefore, the explants will not suffer the overdose.

Conclusion

The present study clearly evaluated the effect of PGRs and culture systems on PLBs induction of Phalaenopsis amabilis leaf explants. Among the PGR treatments, 2 mg.L-1 BA supplemented medium could be recommended as the suitable concentration rate for maximum production of PLBs from leaf explants. Interaction effect was observed between PGRs and culture systems on PLBs induction suggesting that the impact of PGRs could vary depending on the culture systems. These findings highlighted that the effects of plant growth regulators vary with the different culture systems. TDZ should be used as the growth promoter in the solid culture medium and BA should be used in the cotton support liquid culture medium for Phalaenopsis amabilis orchid. Better understanding on the use of

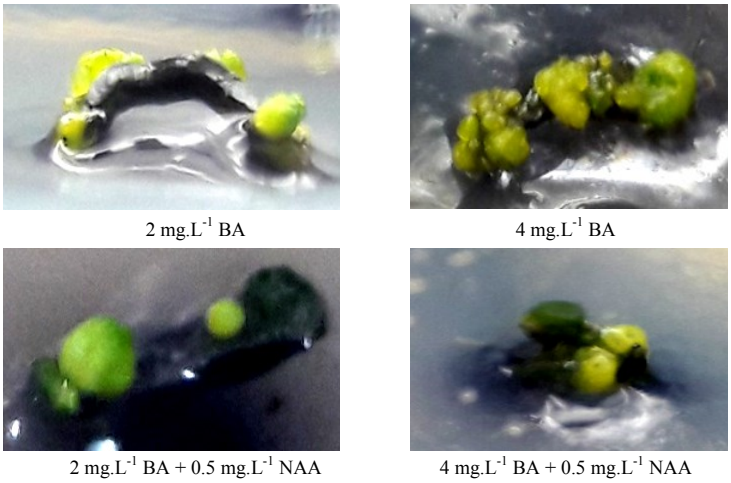

Figure 1. PLBs induction from leaf explants cultured on the solid ½ MS basal medium supplemented with BA hormone

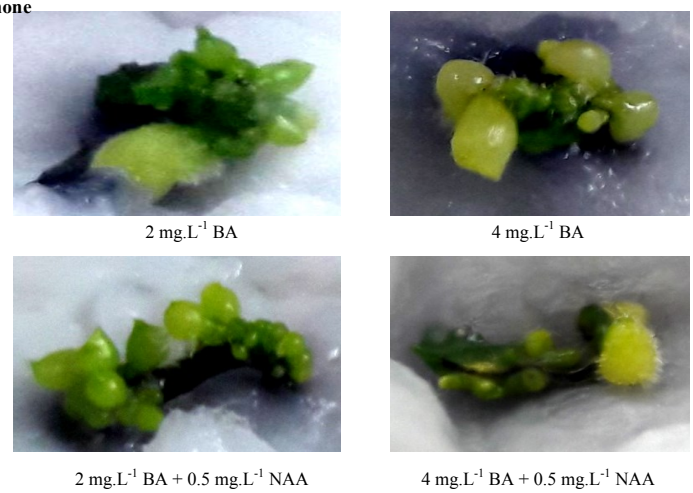

Figure 2. PLBs induction from leaf explants cultured on the cotton ½ MS basal medium supplemented with BA hormone

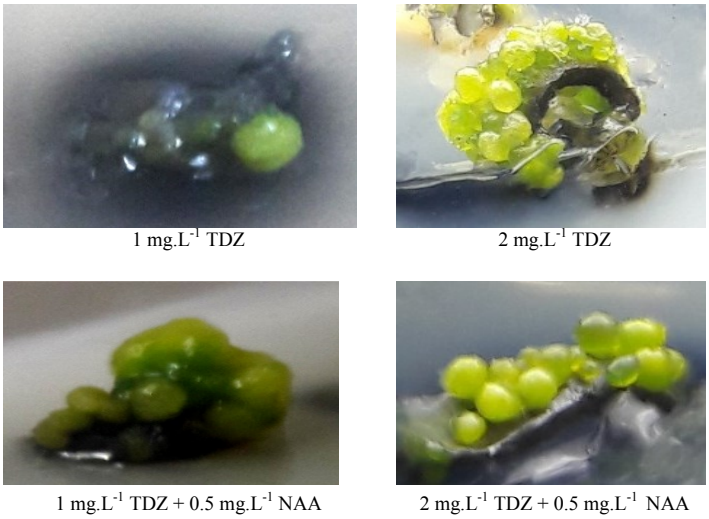

Figure 3. PLBs induction from leaf explants cultured on the solid ½ MS basal medium supplemented with TDZ hormone

different concentrations and the combination effect of these two PGRs in other orchid species are still needed.

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