Sucrose Concentration for Optimum *Phalaenopsis deliciosa* Seedling Growth

L.P. Lim, and C.W. Choong

Abstract**—**High concentration of sucrose may cause seedling dehydration and inactive in autotrophic growth, which causes seedling low survival ability during acclimatization. In order to identify the sucrose concentration for optimum growth, seedlings of *Phalaenopsis deliciosa* was cultured on a defined medium with varying concentration of sucrose. After a culturing period with one subculture in between, shoot and root fresh weight and dry weight were measured to determine growth yield. Types of growths identified were autotrophic, mix autotrophic-heterotrophic and heterotrophic. Sucrose concentration at 20 g/l was identified as optimum concentration, which was the maximum of mix autotrophic-heterotrophic growth range. This concentration is recommended when raising *P. deliciosa* seedlings to retain photosynthetic activity for better survival during acclimatization.

Keywords**—**Optimum sucrose concentration, autotrophic-heterotrophic, *Phalaenopsis deliciosa*, seedling growth, *in vitro* nutrition.

I. Introduction

PHALAENOPSIS *deliciosa* Rchb.f. is an orchid species from the *Phalaenopsis* genus with high ornamental and commercial relevance. It is one of the few genera within the Orchidaceae in which plants bloom every six months, with long lasting inflorescences [1]. However, natural populations of *Phalaenopsis* have been strongly affected due to over-commercialize. In particular, *P. deliciosa* is classified as endangered orchid species in Appendix II of CITES which includes species not necessarily threatened with extinction, but which trade must be controlled in order to avoid utilization which is incompatible with their survival [2].

Micropropagation is one of the technologies used in conservation of threatened plant species [3]. Sucrose is the most common carbon source used in media to allow micropropagation of plant because it is easier to be used and absorbed by tissue culture. However, a continuous supply of sucrose in the culture medium may negatively affect the photosynthetic ability of *in vitro* plants, although appear normal, they may be less likely to be active in autotrophic growth [4]. Another problem arising from the high sucrose content is high osmotic pressure of medium where *in vitro* plants may appear dry and their seed germination could be inhibited [5].

To overcome the photosynthesis inactivation and seed germination problem in plant, sucrose could be reduced or excluded from culture medium to strengthen autotrophic growth and photosynthetic ability of plant. It was found that sucrose nutrition would lead to a mixture of autotrophic and heterotrophic growth, less autotrophic at high sucrose concentration [6]. For orchids, there are different recommendations of sucrose concentration to be used according to different species [7]. Therefore the aim of this study is to investigate sucrose concentration in a defined medium that allows optimum *in vitro* growth of *P. deliciosa*.

II. Materials and Methods

P. deliciosa Rchb.f. was pollinated and the resulting seedpod was collected 110 days after pollination, cleaned with tap water and immersed in 5.25% of sodium hypochlorite for 5 min. Then the seedpod was dipped in 70% ethanol for 1 min followed by brief flaming. The seedpod was cooled briefly, dissected with a scalpel and the seeds inside inoculated on Choong *et al*. (CCT) defined medium [8]. Germinated seedlings were subcultured every 90 days.

Approximately 0.3 ± 0.05 g was inoculated onto CCT media with 0, 6.25, 12.5, 25 and 50 g/l sucrose in 8 replicates. Prior to inoculation, media were sterilized by autoclaving at 121°C for 15 min at 15 psi. The media were dispensed into culture flasks aseptically, approximately 20 ml per flask in a laminar air flow cabinet. After inoculation, seedlings were incubated at 25°C and 16 h photoperiod with photon flux density of 30 μmol m^{-2} s^{-1} for 90 d, with one subculturing at 45 d.

After 90 days of incubation, the seedlings were dissected to shoots and roots and weighed to obtain shoot and root fresh weight. Seedling fresh weight (FW) was calculated by the addition of shoot and root fresh weight. Fresh shoots and roots were dried at 70°C for 2 days and weighed to obtain shoot and root dry weight. Seedling dry weight (DW) was calculated by the addition of shoot and root dry weight. Root to shoot ratio (R/S) was calculated by dividing root dry weight with shoot dry weight.

Data was analyzed as described by [9] where data was tested for normality based on z-score of *kurtosis* and skewness at α = 0.05, analyzed by single-factor analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) two-way pairwise comparisons at α = 0.05. Mix autotrophic-heterotrophic growth range was identified at the region with steepest slope for FW and DW *versus* sucrose concentration curves. Optimum sucrose concentration was

Lei Pin, Lim is with the Faculty of Science, Technology, Engineering and Mathematics (FOSTEM), INTI International University, Persiaran Perdana BBN, Putra Nilai, 71800 Nilai, Negeri Sembilan, Malaysia.

Chieh Wean, Choong is with Faculty of Science, Technology, Engineering and Mathematics (FOSTEM), INTI International University, Persiaran Perdana BBN, Putra Nilai, 71800 Nilai, Negeri Sembilan, Malaysia. (corresponding author's e-mail: chiehwean.choong@newinti.edu.my).

identified as the maximum of mix autotrophic-heterotrophic growth range.

III. Results

As shown in Table 1, treatment means for FW were characterized into three distinct groups, a, b and c. Treatments 25 g/l and 50 g/l sucrose were designated in group a, 12.5 g/l sucrose in group b, 6.25 g/l sucrose in group bc and 0 g/l sucrose in group c. Treatment means for DW were characterized into four groups, a, b, c and d. Treatments 50 g/l sucrose were designated in group a, 25 g/l sucrose in group b, 12.5 g/l sucrose in group c, and 6.25 g/l and 0 g/l sucrose in group d. This indicated that increase in sucrose concentration increased seedling fresh and dry weight.

Treatment means for R/S were characterized into three distinct groups, a, b and c. Treatments 50 g/l sucrose were designated in group a, 25 g/l sucrose in group b, and 12.5 g/l, 6.25 g/l and 0 g/l sucrose in group c. This indicated that increase in sucrose concentration favored root development in the seedlings.

TABLE I
EFFECT OF SUCROSE CONCENTRATION TO SEEDLING YIELD AND ROOT TO SHOOT RATIO

Sucrose concentration (g/l)	FW (g)	DW (g)	R/S
0	0.553 ± 0.071^{c}	0.029 ± 0.005^{d}	2.653 ± 0.765^{c}
6.25	0.691 ± 0.102^{bc}	0.041 ± 0.005^{d}	2.179 ± 0.179^{c}
12.5	0.800 ± 0.086^{b}	0.057 ± 0.007^{c}	2.835 ± 0.478^{c}
25	1.031 ± 0.169^{a}	0.094 ± 0.017^{b}	4.733 ± 0.585^{b}
50	0.989 ± 0.076^{a}	0.137 ± 0.009^{a}	9.604 ± 1.731^{a}

Effect of sucrose concentration on mean of *Phalaenopsis deliciosa* Rchb.f. seedling fresh weight (FW), dry weight (DW) and root to shoot ratio (R/S) with respective 95% confidence intervals. Treatments were carried out at 8 replicates for 90 days with subculturing after 45 days. Treatment means were analyzed by single-factor analysis of variance and two-way pairwise comparisons between those treatment means were performed with Fisher's LSD test at $\alpha = 0.05$ that separated treatment means with significant difference ($\rho \geq 0.95$) into distinct groups represented by small caps alphabets.

The curves for FW and DW (Figure 1) have a distinct turn where the slope at its steepest suddenly reduced at approximately 20 g/l sucrose. Autotrophic growth phase was predicted from 0 g/l to 12.5 g/l sucrose, mix autotrophic-heterotrophic growth phase (the steepest portion) from 12.5 g/l to 20 g/l sucrose, and heterotrophic growth phase from 20 g/l to 50 g/l sucrose. The maximum value of mix autotrophic-heterotrophic growth phase was identified approximately at 20 g/l.

IV. Discussion

When inspecting water content by comparing the curve of FW and DW *versus* sucrose concentration, low hydration levels were observed with seedlings treated with 25 g/l and 50 g/l sucrose. This could be due to high osmotic pressure of the media. Sucrose concentration significantly contributes to the osmotic pressure of culture media [4]. Therefore, sucrose concentration of below 25 g/l should be used when culturing *P. deliciosa* seedlings.

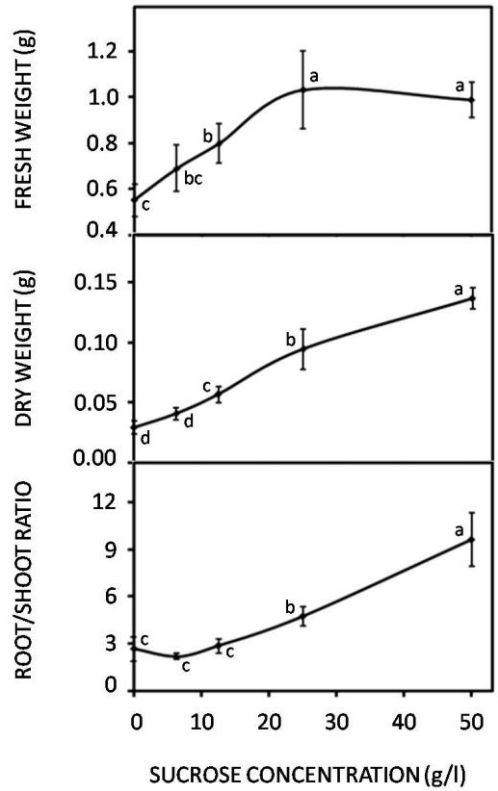

Fig. 1. Effect of sucrose concentration (0, 6.25, 12.5, 25 and 50 g/l) on means of *Phalaenopsis deliciosa* Rchb.f. seedling ($n = 40$; $n = 8$ per treatment) fresh weight (FW), dry weight (DW) and root to shoot ratio (R/S) with 95% confidence interval error bars. Treatment means with significant difference ($\rho \geq 0.95$) were separated into distinct groups represented by small cap alphabets.

In addition, the results led to the identification of 20 g/l sucrose as the maximum for mix autotrophic-heterotrophic growth. This is the concentration predicted to retain photosynthetic ability of cultured *P. deliciosa* seedlings. Excessive amount of sucrose in culture medium encouraged mainly heterotrophic growth [6] and may inhibit chlorophyll formation and inactivate the enzyme responsible for photosynthesis ability of *in vitro* plants, causing less autotrophic growth [4]. Even though heterotrophic *in vitro* seedlings may appear normal, they are less likely to be active in autotrophic growth and less adaptable to acclimatization [4].

Based on the literature study that investigated the effects of sucrose concentration on growth of *Calanthe* (orchid) hybrids, 'Bukduseong' × 'Hyesung' [10], after 8 week of culturing period, the fresh weight and dry weight of shoot were found to be highest at sucrose level of 15 g/l and decreased at higher sucrose treatment that give rise to more root growth. This study supported 15 g/l as optimal for *Calanthe* hybrid in [10] study, since 15 g/l is within the mix-autotrophic growth phase identified in this study.

Besides, according to the literature finding related to the seed of *Epidendrum* and *Cattleya* orchid species [11], shoot

growth was noticed to be decreased at higher sucrose concentrations which increased root growth. The finding matched with results of this study, where less shoot growth and more root growth were obtained when seedlings were treated with higher than 20 g/l sucrose.

V. Conclusion

Optimum growth would be obtained at the maximum of mix autotrophic-heterotrophic growth phase which was identified at 20 g/l. Concentration of sucrose higher than 20 g/l was predicted as heterotrophic growth phase while concentration of sucrose lower than 12.5 g/l was predicted to be autotrophic growth phase. As sucrose concentration increased, more increase in root growth than shoot growth was measured. Culturing *P. deliciosa* at 20 g/l is recommended to produce autotrophic active seedlings with increased survival during acclimatization.

Acknowledgment

This study was financially supported by and conducted at INTI International University, Nilai campus.

References

[1] A.R. Tavares, J.L.M. Young, S.S. Ori, S. Kanashiro, G.P. Lima, E.P. Chu and R.M. Suzuki, "Orchid *in vitro* growth as affected by nitrogen levels in the culture medium," *Horticulturae Brasileira*, vol. 30, pp. 119–124, 2012.

[2] CITES, *The Cites Appendices*, n.d., retrieved from http://www.cites.org/eng/app/

[3] V. Sarasan, R. Cripps, M.M. Ramsay, C. Atherton, M. McMichen, G. Prendergast and J.K. Rowntree, "Conservation *in vitro* of threatened plants – progress in the past decade," *In Vitro Cell and Developmental Biolology—Plant*, vol. 42, pp. 206-214, 2006.

[4] E.F. George and G.J. De Klerk, "The Components of Plant Tissue Culture Media II: Organic Addictions, Osmotic and pH Effects, and Support Systems," in *Plant Propagation by Tissue Culture*, E.F. George, M.A. Hall and G.J. de Klerk, Eds. Dordrecht, Netherlands: Springer, 2008, pp. 65-113.

[5] M. Evenari, "Germination inhibitors," *Botanical Review*, vol. 15, pp. 153–194, 1949.

[6] S. Wolf, N. Kalman-Rotem, D. Yakir and M. Ziv, "Autotrophic and heterotrophic carbon assimilation of *in vitro* grown potato (*Solanum tuberosum* L.)" *Journal of Plant Physiology*, vol. 135, pp. 574-580, 1998.

[7] J. Arditti and R. Ernst, *Micropropagation of Orchids*, 2nd ed., Oxford, UK: Wiley-Blackwell Basingstoke, 1992.

[8] C.W. Choong, S.M. Choong and W.H. Thong, "A defined culture medium suitable for sensitive *Phalaenopsis* species seedlings," *Biotechnology*, vol. 12, no. 3, pp. 162-167, 2013.

[9] C.W. Choong and S.M. Choong, "Optimum nitrogen to sulfur ratio to increase *in vitro* seedling yield of *Phalaenopsis bellina*," *International Journal of Chemical, Environmental and Biological Science (IJCEBS)*, vol. 1, no. 5, pp. 796-797, 2013.

[10] M.A. Baque, Y.K. Shin, T. Elshmari, E.J. Lee and K.Y. Paeki, "Effect of light quality, sucrose and coconut water concentration on the microporpagation of *Calanthe* hybrids ('Bukduseong' × 'Hyesung' and 'Chunkwang' × 'Hyesung')," *Australian Journal of Crop Science*, vol. 5, no. 10, pp. 1247-1254, 2011.

[11] R.C. Yates and J.T. Curtis, "The effect of sucrose and other factors on the shoot-root ratio of orchid seedlings. *American Journal of Botany*, vol. 36, no. 5, pp. 390-396, 1949.