Effects of Different LED Lights on the Organogenesis of a *Cymbidium* Cultivar

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We examined the effects of LED light spectrum and alternate lighting on the proliferation of protocorm-like bodies (PLBs) and formation of shoot and root on explants of *Cymbidium* Sweet Waffle ‘Tarte’ in order to find the optimal lighting condition for micropropagation of the orchid. We maintained the treatments at 25±1°C for 24 hours light period. We found that proliferation of PLBs was increased most under monochromatic green LED light (peak wavelength: 517 nm) and alternate green LED light and red LED light (peak wavelength: 631 nm). We also found that shoot formation was increased most under monochromatic red LED light and alternate green LED light and red LED light. Root formation was increased most under blue LED light (peak wavelength: 460 nm). Based on these findings, we concluded that alternate green LED light and red LED light is the optimal lighting condition, because PLB proliferation and fresh weight increase are important for micropropagation of orchids.

Keywords: *Cymbidium*, light-emitting diodes (LEDs), organogenesis, protocorm-like bodies (PLBs), wavelength

INTRODUCTION

Artificial light is a primary source of energy which influence the morphogenesis and growth of plant cell, tissue and organ in tissue culture techniques (Reuveni and Evenor, 2007). For commercial micropropagation, using LEDs as a radiation source is increasing. To find out a optimum lighting condition is very important for mass propagation of orchids. The effects of LED lights were studied in various orchid species (Anuchai and Hsieh, 2017; Kaewjampa and Shimasaki, 2012; Mengxi et al., 2011; Ramírez-Mosqueda et al., 2017). But most of the studies focus on red and blue LEDs. Hence, this study was taken to investigate effect of monochromatic green LED, and alternating lighting condition of red, blue and green LEDs on PLB proliferation, fresh weight, shoot and root formation by protocorm-like bodies of a *Cymbidium* cultivar.

MATERIALS AND METHODS

Plant materials

PLBs (average fresh weight 30 mg) of *Cymbidium* Sweet Waffle ‘Tarte’ were used as explants. This cultivar was provided by Mr. Masanori Ujike, company president of Bio-U, Zentsuji, Kagawa, Japan. The cultivar is a hybrid of *Cymbidium* Great Katy and *Cymbidium* Stellar Festival.

Culture medium

PLBs were cultured in MS medium (Murashige and Skoog, 1962) supplemented with 412.5 mg L⁻¹ NH₄NO₃, 950 mg L⁻¹ KNO₃, 20 g L⁻¹ sucrose, 2.2 g L⁻¹ Phytagel, and 1mM 4-Morpholineethanesulfonic acid sodium salt (MES Na) (Sigma Aldrich Co., USA). No plant growth regulator was used in the media. To obtain desired number of PLBs, they were proliferated by transferring the PLBs to new medium every two months. The media were adjusted to pH 5.5–5.8 and sterilized by autoclaving at 121°C for 15 minutes. Culture bottles (UM culture jar, 250 mL; AsOne, Japan) with plastic caps were used. Each bottle received 30 ml of medium.

Lighting source

To determine the effect of LED lights on the organogenesis, the cultures were established and grown under different lighting conditions at a photon flux density (PFD) of 40 μmol m⁻² s⁻¹. This PFD value was found optimum from our previous study (Unpublished). There were eight treatments: (1) FL: white fluorescent tube (National FL20SS,) as control, (2) B: blue LED (LT 20B 9W 1447, peak wavelength: 460 nm), (3) R: red LED (LT 20R 9W 1449, peak wavelength: 631 nm), (4) G: green LED (LT 20G 9W 1524, peak wavelength: 517 nm), (5) W: white LED (LT L20KY 9W 1532, peak wavelength: 454 nm), (6) R+B: alternating red and blue LED at 24 hours intervals, (7) G+B: alternating green and blue LED at 24-hour intervals and (8) G+R: alternating green and red LED at 24-hour intervals. For treatments 6–8, different LED lights were used at 24-hour intervals in an alternating pattern. All treatments were maintained at 25±1°C continuously for 24 hours/day over 7 weeks (49 days). Emission spectra of dif-
Different LED lights were measured by using a light analyzer (LA-105; NK System, Osaka, Japan) (Fig. 1).

Data collection and statistical analysis
Experimental data were collected after 49 days of culture by counting the average number of new PLBs, shoots, and roots as in Fig. 2. The formation ratio (%) of the number of PLBs, shoots, and roots to the number of explant were recorded. The total fresh weight (FW) of PLBs per inoculated explant was measured. Number of leaves per shoot and shoot forming root ratio were counted. Five explants were put in each culture vessel and three culture vessels were used for one replication, that is, 15 explants were used for one replication. The formation ratio of PLBs, shoots and roots for each replication were calculated.

Fig. 1 Emission spectra of white fluorescent tube (A), blue (B), red (C), green (D) and white LED (E) lights used in the experiment. This figure was made using light analyzer PC software. The photon flux density (PFD) and wavelength were measured using a light analyzer (LA-105; NK System, Osaka, Japan).

Fig. 2 Data collection method. P: average number of PLBs per explant, S: average number of shoots per explant, R: average number of roots per shoot for each culture vessel.
as follows: Formation ratio of PLBs/shoots/roots (%) = n/15x100 where, n = 0 or 1 (0, No new PLBs/shoots/roots, 1, new PLBs/shoots per inoculated explant or new roots/shoot)

The calculation of formation ratio of PLBs, shoots, or roots represents whether or not PLBs, shoots, or roots generated from an explant, respectively.

The experiment was set up in a completely randomized design with five replications. Statistical evaluation was done by using one-way analysis of variance (ANOVA) and then analyzed with Tukey’s honestly significant difference test (Tukey’s HSD) at P < 0.05 using KaleidaGraph-4.5.0 (Synenergy Software, USA) to test for significant differences among the sample means.

RESULTS AND DISCUSSION

Effect of different LED lights on the PLB formation and fresh weight

Table 1 shows after 7 weeks of culture, PLB proliferation and fresh weight (mg) was significantly affected by the different LED lights in vitro. The average number of PLBs was found highest under G (44.3) followed by G+R (32.2). Formation ratio of PLBs was obtained 100% with G and G+R. Larger PLBs (>2 mm in length) was obtained 100% with G. G+R resulted 80% formation of larger PLBs (>2 mm in length). The lowest number of PLBs (3.5/ explant) and formation ratio of PLBs (20%) was produced with FL (Table 1). There was no formation of larger PLBs (>2 mm in length) with FL. Physical appearances of PLBs under different light sources are shown in Fig. 3. The highest total fresh weight (1323.3 mg) was with R. G+R resulted 913 mg of fresh weight which was about 10 times more than FL (99.3 mg). So our result showed that, both monochromic green LED and alternating of green and red LEDs had a promising effect on PLB formation and fresh weight than other lighting treatments (Table 1).

Kaewjampa and Shimasaki (2012) also found that adding monochromic green LED and the alternating of green and blue light, which could affect the morphogenesis in orchids (Islam et al., 1999; Vogel and Macedo, 2011). Green LEDs is beneficial for PLB formation in orchid in vitro cultivation (Alvarenga et al., 2015; Ramírez-Mosqueda et al., 2017). Phytochrome and chryptochrome absorb the spectra of red and blue light, respectively. According to Kwon et al. (2015) there is a synergy relation between phytochrome and chryptochrome which could affect the morphogenesis in in vitro condition. Manivannan et al. (2015) showed that red LED stimulated endogenous gibberellins involved in mitosis and cell

| Lighting source | Average number per explant | Formation ratio (%) | Total fresh weight per explant (mg) | Average number per explant | Formation ratio (%) | No. of leaves per shoot | Average number per shoot | Formation ratio (%) | Shoot forming root ratio (%) |
|-----------------|----------------------------|---------------------|-------------------------------------|----------------------------|---------------------|--------------------------|-------------------------|---------------------|-----------------------------|
| FL              | 3.3±                      | 20                  | 99.3±                               | 2.0±                      | 67                  | 1.9±                     | 0.5±                    | 87                  | 23                          |
| W               | 6.3±                      | 40                  | 200.2±                              | 2.9±                      | 67                  | 1.8±                     | 1.0±                    | 73                  | 40                          |
| R               | 18.3±                     | 73                  | 1323.3±                             | 7.5±                      | 100                 | 3.2±                     | 1.5±                    | 93                  | 50                          |
| B               | 10.1±                     | 60                  | 276.3±                              | 3.8±                      | 53                  | 2.1±                     | 2.9±                    | 100                 | 73                          |
| G               | 44.3±                     | 100                 | 372.0±                              | 2.0±                      | 33                  | 0.7±                     | 0.2±                    | 20                  | 27                          |
| B+R            | 10.6±                     | 73                  | 731.3±                              | 5.0±                      | 73                  | 2.8±                     | 1.7±                    | 80                  | 90                          |
| G+B            | 24.6±                     | 80                  | 401.0±                              | 3.1±                      | 40                  | 1.0±                     | 0.7±                    | 67                  | 47                          |
| G+R            | 32.3±                     | 100                 | 913.3±                              | 5.7±                      | 80                  | 1.7±                     | 0.4±                    | 67                  | 33                          |

FL: white fluorescent light, W: white LED, R: red LED, B: blue LED, G: green LED, R+B: alternating red and blue LED at 24-hour intervals, G+B: alternating green and blue LED at 24-hour intervals, G+R: alternating green and red LED at 24-hour intervals. Mean values that do not share a letter are significantly different within each column, and those sharing a letter are statistically similar by Tukey’s HSD test (P < 0.05).
proliferation. Tanaka et al. (1998) also found that red LED enhanced the leaf growth but decreased chlorophyll content, which was reversed by blue LEDs by using super bright blue and red LEDs in Cymbidium plantlets. On the contrary of our result, Kaewjampa and Shimasaki (2012) found that, additional green LED with blue LED increased shoot formation in Cymbidium Waltz 'Idol'. This variation may occur due to genetic attributes and requirement of light photoreceptor varied among species and cultivars (Folta and Maruhnich, 2007). Plants possess a complex and dynamic light response and memory system that involves reactive oxygen species and hormonal signaling, which are used to optimize light acclimation and immune defenses (Szczypinska-Hebda et al., 2010). Root formation may be affected by the hydrogen peroxide (Cao et al., 2014). Blue light activated different defensive system to reduce excessive amounts of reactive oxygen species (Mengxi et al., 2011). The effects of LED lights on the organogenesis vary among orchid species (Tanaka et al., 1998, Mengxi et al., 2011; Kaewjampa and Shimasaki, 2012, Ramirez-Mosqueda et al., 2017). Studies compare only specific light ratios in different species which make difficulties to understand plant’s response to particular light because their responses are often contradictory (Kihori and Myung-Min, 2013, Wojciechowska et al., 2016). However, the response of cultured explants to different light conditions might be dependent on species or even clonal specificities as well as specific light conditions (Huan and Tanaka, 2004). Our study confirmed that, LED lights produce differential effects on the organogenesis of Cymbidium. Here, both green and alternating green and red LED showed optimum PLB formation and fresh weight. Monochromic red LED increased the shoot formation, monochromic blue LED enhanced the root formation but alternating blue and red LED resulted maximum percentage of shoot forming roots. In orchid micropropagation, multiple shoot formation provides the production of uniform seedlings and reduces the occurrence of somaclonal variation (Jainul and Jualang, 2015). Some orchid growers give emphasis on the percentage of shoot forming root for its commercial cultivation. Plantlets with high root formation can maintain the internal water and absorb nutrients easily, which is suitable for acclimatization and successfully survive in greenhouse conditions (Mohanty et al., 2012). As the generation of protocorm-like bodies (PLBs) and fresh weight is an important technique for micropropagation of orchids (Arditti and Ernst, 1993), our findings suggest that, alternating green and red LED light is beneficial for micropropagation of Cymbidium cultivar.

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REFERENCES

Anuchai, J., Hoeie, C. H. 2017. Effect of change in light quality on physiological transformation of in vitro Phalaenopsis ‘Fortune Saltzman’ seedlings during the growth period. Hort. J. 86: 395–402.

Arditti, J., Ernst, R. 1993. Micropropagation of Orchids. John
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Wiley and Sons, New York.
Alvarenga, I. C. A., Pacheco, F. V., Silva, S. T., Bertolucci, S. K. V., Pinto, J. E. B. P. 2015. In vitro culture of Achillea mil- lefolium L.: quality and intensity of light on growth and produc- tion of volatiles. Plant Cell Tiss. Org. Cult. 122: 299-308.
Cao, Z., Fang, T., Chen, M., Li, J., Shen, W., Huang, L. 2014. Involvement of haem oxygenase-1 in hydrogen peroxide-in- deduced lateral root formation in tomato. Acta Physiol. Plant. 36: 931-943.
Folta, K. M., Maruhnich, S. A. 2007. Green light: a signal to slow down or stop. J. Exp. Bot. 58: 3099-3111.
Huan, L. V. T., Tanaka, M. 2004. Effects of red and blue light-emitting diodes on callus induction, callus proliferation, and protocorm-like body formation from callus in Cymbidium orchid. Environ. Control Biol. 42: 57-64.
Islam, M. O., Matsu, S., Ichihashi, S. 1999. Effects of light quality on seed germination and seedling growth of Cattleya orchids in vitro. Jpn. Soc. Hortic. Sci. 68: 1132-1138.
Jamil, J. E., Jualang, A. G. 2015. In vitro shoot multiplication and rooting of shoot tip explants of Dimorphorchis lowii: an endemic orchid of Borneo. J. Trop. Plant Physiol. 7: 14-25.
Kawamiya, N., Shimasaki, K. 2012. Effects of green LED lighting on organogenesis and superoxide dismutase (SOD) activities in protocorm-like bodies (PLBs) of Cymbidium cultured in vitro. Envron. Control Biol. 50: 247-254.
Kim, S., Myung-Min, O. 2013. Leaf shape, growth, and anti- oxidant phenolic compounds of two lettuce cultivars grown under various combinations of blue and red light-emitting diodes. HortScience 48: 988-995.
Kwon, A. R., Cui, H. Y., Lee, H. 2015. Light quality affects shoot regeneration, cell division, and wood formation in elite clones of Populus euramericana. Acta Physiol. Plant. 37: 65.
Manivannan, A., Soundararajan, P., Halimah, N., Ko, C. H. 2015. Blue LED light enhances growth, phytochemical contents, and antioxidant enzyme activities of Rehmannia glutinosa cultured in vitro. Hortic. Environ. Biotechnol. 56: 105-113.
Mengxi, L., Zhigang, X., Yang, Y., Yijie, F. 2011. Effects of dif- ferent spectral lights on Oncidium PLBs induction, proliferation, and plant regeneration. Plant Cell Tiss. Org. Cult. 106: 1-10.
Mohanty, P., Paul, S., Das, M. C., Kumaria, S., Tanon, P. 2012. A simple and efficient protocol for mass propagation of Cym- bidium mastersii: an ornamental orchid of Northeast India. AoB PLANTS: pls023.
Murasuhi, T., Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15: 473-497.
Okazaki, S., Yamashita, T. 2019. A manipulation of air tempera- ture and light quality and intensity can maximize growth and folate biosynthesis in leaf lettuce. Environ. Control Biol. 57: 39-44.
Ramirez-Mosqueda, M. A., Iglesias-Andreu, L. G., Luna-Sánchez, I. J. 2017. Light quality affects growth and development of in vitro plantlet of Vanilla planifolia Jacks. S. Afr. J. Bot. 109: 288-293.
Reuveni, M., Evenor, D. 2007. On the effect of light on shoot regeneration in petunia. Plant Cell Tiss. Org. Cult. 89: 49-54.
Sarropoulou, V., Maloupa, E. 2012. Effects of red and blue light- emitting diodes on rooting rate and shoot formation of Cymbidium cultured under superbright red and blue light-emitting diodes (LEDs). J. Hortic. Sci. Biotechnol. 78: 247-254.
Wojciechowska, R., Kurpaska, S., Malinowski, M. 2010. Evidence for light wavelength-specific systemic photoelectrophysiological signalling and cellular light memory of excess light episode in Arabidopsis. Plant Cell 22: 2201-2218.
Tanaka, M., Takamura, T., Watanabe, H., Endo, M., Yanagi, T., Okamoto, K. 1998. In vitro growth of Cymbidium plantlets cultured under superbright red and blue light-emitting diodes (LEDs). J. Hortic. Sci. Biotechnol. 73: 39-44.
Vogel, I. N., Macedo, A. F. 2011. Influence of IAA, TDZ, and light quality on asymmetric germination, protocorm formation, and plantlet development of Cyrtopodium glutiniferum Radd., a medicinal orchid. Plant Cell Tiss. Org. Cult. 104: 147-155.
Wojciechowska, R., Kurpaska, S., Malinowski, M. 2016. Effect of supplemental LED lighting on growth and quality of Vale- rianella locusta L. and economic aspects of cultivation in autumn cycle. Acta Sci. Pol. Hortorum Cultus 15: 233-244.