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Effects of sucrose and trehalose on growth and development of protocorm-like bodies (PLBs) on two *Dendrobium* cultivars under different lights

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ABSTRACT

The objective of this study was to identify effective carbon sources for the in vitro propagation of PLBs in Dendrobium cultivars Dendrobium kingianum 'Hallelujah' and Dendrobium k. Jonathan's Glory 'Dark Joy'. In this study, we used two types of carbon sources to culture the cultivars under five different LED lights. For, both carbon sources the highest numbers of PLBs were obtained with the green LEDs compared with the other LED lights. For Dendrobium kingianum 'Hallelujah' the trehalose supplemented medium produced the highest number of PLBs (13.8/explant) and the maximum fresh weight (0.45g/explant) under green LEDs. On the other hand, for Dendrobium k. Jonathan's Glory 'Dark Joy', the sucrose supplemented medium produced the highest number of PLBs under green LED (8.0/explant, fresh weight 0.22g/explants) and the trehalose supplemented medium produced the maximum number of PLBs under white LEDs (8.1/explants, fresh weight 0.23g/explants). The sucrose was the most relevant carbon source for the in vitro organogenesis of Dendrobium k. Jonathan's Glory 'Dark Joy', while trehalose was best under white LEDs.

Key Words*: Dendrobium, protocorm-like bodies (PLBs), Light emitting diodes (LEDs)*

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I. Introduction

Orchids are grown as exquisite and are very popular as cut flowers in the flower industry. Chugh et al. (2009) reported that multiplication of orchids, especially rare hybrids and endangered species using tissue culture techniques has encouraged orchids to occupy a position as one of the top ten cut flowers. Among orchids, *Dendrobiums* with their profuse, delicate blooms are hugely popular. In comparison with plantlet development from seeds or adventitious shoots, micropropagation through PLBs is more capable because PLBs can be swiftly proliferated on solid or in liquid culture medium, and a large number of PLBs can be produced in a short period (Luo et al., 2003). Studies have revealed

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that the optimization of medium composition is an important approach in improving the micropropagation process of orchids by culturing PLBs that are species-specific (Shimura and Koda, 2004; Luo et al., 2009). The prosperity of plant tissue culture is influenced by media composition. Media used for tissue culture of orchids is generally high in salt, minerals, vitamins, growth regulators and water (Murdad et al. 2010). Plant tissue culture, which is appropriate for orchid production, requires a continuous supply of carbohydrates to ensure plant growth and survival (Paiva-Neto and Otoni, 2003). In-plant tissue culture media, the carbon source is very important because it supplies energy to the plants especially when they are not ready to photosynthesize during the initial stage of tissue culture (Al-Khateeb, 2008). Sucrose has been recommended as the most suitable source of carbon and energy for propagation of *in vitro* plants (Paiva-Neto and Otoni, 2003, Kane, 2011). Sucrose is easily available to cells and directly participates in glycolytic and pentose phosphate pathways for cell growth and was also found to have an osmotic role in the culture system (Zha et al., 2007). In our study, we used trehalose (a-D-glucopyranosyl-(1,1)-a-D-glucopyranoside) as a substitute for a carbon source for the proliferation of PLBs. Trehalose is a nonreducing sugar, not easily hydrolyzed by acid, and the glycosidic bond is not sunder by alpha-glucosidase. Trehalose has been reported to be used for orchid seed propagation (Ernest, 1967; Liu et al., 2006; Smith, 1973) and was compared with different sugars as an energy source for symbiotic germination. Environmental factors are also responsible for modifying plant development. Light is one important abiotic factor for orchid micropropagation *in vitro*. Light also affects PLB regeneration through photosynthetic and phototropic responses which may depend on light quality and photoperiod (Taiz and Zeiger 1991). Fluorescent lights are commonly used in plant tissue culture but in recent years, LEDs have been applied as the sole source of lighting. Compared with fluorescent light, LED lighting reform the quality of orchid plantlets obtained via tissue culture technology. Fluorescent lights consume more power than LEDs, and they produce a wide range of wavelengths (350–750 nm) that are unnecessary for plant development, whereas monochromatic LEDs emit light at specific wavelengths.

An *in vitro* environment and externally applied pressures influence the regeneration ability of tissue culture in plants. The objective of the study was to determine the response of the application of sucrose and trehalose on *Dendrobium kingianum* **'**Hallelujah**'** and *Dendrobium k.* Jonathan's Glory 'Dark Joy' using different light emitting diodes (LEDs) and to identify the most effective light source for the rapid multiplication of orchids.

II. Materials and Methods

Plant and culture conditions

This study on *D. kingianum* 'Hallelujah' and *Dendrobium k.* Jonathan's Glory 'Dark Joy' was conducted from March, 2019 to January, 2020 in the Laboratory of Floriculture & Vegetable Science, Faculty of Agriculture, Kochi University, Japan. Protocorm-like bodies (PLBs) were subcultured every two months in modified Murashige & Skoog (MS) medium (Shimasaki and Uemoto, 1990), supplemented with 412.5 mg/L ammonium nitrate, 950 mg /L potassium nitrate, and 2 g/L Phytagel (Sigma). In this study, 20g /L each of sucrose and trehalose was used separately as carbon sources. Modified MS medium was adjusted to pH 5.5- 5.8 with 1 mM 2-(N-morpholino) ethanesulfonic acid sodium salts (MES-Na) before autoclaving at 121 °C for 15 min at 1.5 Kgf cm^2 UM culture bottles (250 mL; AsOne, Japan) with plastic caps were used, with each bottle receiving 30 mL of medium. Five explants were put in each culture vessel and three culture vessels were used for each treatment.

Lighting conditions

To determine the effective light sources for PLB propagation of the *Dendrobium* cultivars *in vitro*, PLBs were cultured under different light conditions with a photon flux density (PFD) of 54 µmol m^2, S^1. Five sources of light were used in this study: white fluorescent lamp (18W 1XFL20SS/18 as control; National), red LEDs (LT20R 9W 1449; Beamtech), blue LEDs (LT20B 9W 1447; Beamtech), green LEDs (LT20GS 9W 1524; Beamtech), and white LEDs (LTL T20KY 9W 1532; Beamtech). All cultured were maintained at 25±1^0 Cand 24 h photoperiods for six weeks.

Data collection and analysis

After six weeks of cultures, data were collected by counting the number of PLBs, shoots, roots, and their fresh weight was also measured. The means and standard errors (SE) were calculated and

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expressed as means ±SE. The data were statistically analyzed by using Tukey HSD tests at the 5 % level.

III. Results

Effects of sucrose and trehalose on *Dendrobium kingianum* 'Hallelujah' under different light conditions

The results of the *in vitro* culture of protocorm-like bodies (PLBs) of *D. kingianum* 'Hallelujah' varied significantly under the different LEDs. Data obtained after six weeks of culture show that the numbers of PLBs were statistically significant for all LEDs but were statistically different from control (Table 01). However, the green LED light produced the highest number of PLBs in both the sucrose and trehalose treatments (13.8 and 11.1/ explant, respectively) (Table 01). The white fluorescent lamps (control) resulted in the lowest number of PLBs per explant for both the sucrose and trehalose treatments and the lowest formation percentage (93%) (Table 01). The maximum PLB formation rate of (100%) and the highest fresh weight were attained with the green LED light in the culture media treated with both sucrose and trehalose separately (Table 01). No roots were generated within the period of culture to use of sucrose in this study (Figure 01). The *in vitro* sucrose and trehalose supplemented culture media under green LED stimulated the PLBs organogenesis of *D. kingianum* 'Hallelujah'.

Table 01. Effects of sucrose and trehalose on *Dendrobium kingianum* 'Hallelujah' under different light conditions

Carbon source 20g/L	Light condition	No. of PLBs/ explants	% of PLBs	No. of shoots	No. of roots	Fresh weight (g)
Sucrose	White fluorescent lamp	6.1±0.45^{b}	93	1.27±0.12^{a}	-------	0.21±0.01^{ab}
	White LED	9.8±0.26^{a}	100	0.4±0.06^{ab}	-------	0.35±0.01^{a}
	Blue LED	9.3±0.22^{a}	100	-------	-------	0.35±0.01^{a}
	Red LED	11.0±0.27^{a}	100	-------	-------	0.44±0.01^{a}
	Green LED	11.1±0.30^{a}	100	-------	-------	0.45±0.02^{a}
Trehalose	White fluorescent lamp	7.4±4.06^{b}	93	0.86±1.4^{a}	0.53±1.1^{a}	0.22±0.09^{c}
	White LED	10.3±4.1^{ab}	100	-------	-------	0.37±0.12^{a}
	Blue LED	11.6±3.3^{a}	100	-------	-------	0.35±0.11^{a}
	Red LED	11.8±3.8^{a}	100	-------	-------	0.27±0.11^{b}
	Green LED	13.8±4.5^{a}	100	-------	-------	0.42±0.16^{a}

Different letters in the same column indicate significant differences using Tukey HSD tests at the 5% level.

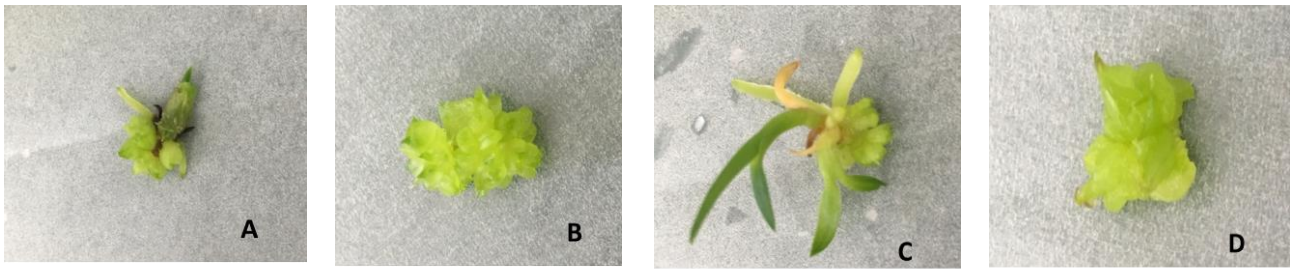

Figure 01. Effects of sucrose and trehalose on *Dendrobium kingianum* 'Hallelujah' under different LEDs. A: sucrose and white fluorescent lamp; B: sucrose and green LED; C: trehalose and white fluorescent lamp; D: trehalose and green LED.

Effects of sucrose and trehalose on *Dendrobium kingianum* Jonathan's Glory 'Dark Joy' under different light conditions

The sucrose supplemented culture medium produced the maximum average number of PLBs (8.0/explant) and the highest number of shoots (2.6/explant) under green LED light for *Dendrobium k.* Jonathan's Glory 'Dark Joy' (Table 02). The maximum fresh weight (0.22g) and maximum percentage of PLB formation (100%) were also observed with the same treatment (Table 02). On the other hand, the trehalose supplemented medium under white LED lights increased the average number of PLBs with 100% PLB formation rate (Table 02). Green LEDs produced the lowest number of PLBs

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(4.1/explant) and the lowest PLB formation (87%), which were lower than for the white fluorescent lamps (control). The *in vitro* PLBs organogenesis of *Dendrobium k.* Jonathan's Glory 'Dark Joy' was stimulated by the sucrose supplemented media under green LEDs. Whereas the organogenesis was stimulated by the trehalose supplemented medium under white LEDs.

The results showed the effects of sucrose and trehalose used separately as carbon sources on *Dendrobium k*. Jonathan's Glory 'Dark Joy' under different LEDs. Table 02, shows that the PLBs of *Dendrobium k.* Jonathan's Glory 'Dark Joy' responded positively under different LEDs lights with sucrose used as the carbon source in the culture vessels. On the other hand, with trehalose in the culture media, the response of the PLBs of this cultivar was comparatively low. In the sucrose medium, the maximum average number of PLBs (8.0/explant) and shoots (2.6/explant) were produced under the green LED light (Table 02). The fresh weight was highest with the same treatment and shoot formation also showed better results in this cultivar (Figure 02). On the other hand, in the trehalose medium, the white LED lights increased PLB formation, but the other LED lights reduced the average number of PLBs compared with the white fluorescent lamps.

Table 02. Effects of sucrose and trehalose on *Dendrobium kingianum* Jonathan's Glory 'Dark Joy' under different LEDs

Carbon source 20g/L	Light condition	No. of PLBs/explants	% of PLBs	No. of shoots	No. of roots	Fresh weight (g)
Sucrose	White fluorescent lamp	5.3±2.1^{b}	100	1.9±2.3^{a}	--------	0.17±0.1^{ab}
	White LED	6.9±2.7^{ab}	100	0.7±1.4^{a}	0.1±0.4^{a}	0.13±0.1^{bc}
	Blue LED	6.1±2.7^{ab}	86	1.8±2.2^{a}	0.5±1.1^{a}	0.18±0.1^{ab}
	Red LED	5.4±1.3^{b}	100	0.8±1.1^{a}	0.1±0.3^{a}	0.10±0.1^{c}
	Green LED	8.0±2.9^{a}	100	2.6±1.9^{a}	0.1±0.3^{a}	0.22±0.1^{a}
Trehalose	White fluorescent lamp	6.9±3.1^{a}	100	0.8±1.4^{a}	---------	0.14±0.1^{ab}
	White LED	8.1±2.9^{a}	100	1.6±1.1^{a}	0.1±0.5^{a}	0.23±0.2^{a}
	Blue LED	5.0±4.1^{ab}	93	1.3±1.6^{a}	0.8±1.2^{a}	0.14±0.1^{ab}
	Red LED	5.2±3.0^{ab}	100	1.4±1.5^{a}	0.5±0.8^{a}	0.15±0.1^{ab}
	Green LED	4.1±2.7^{b}	87	1.3±1.2^{a}	0.5±0.7^{ab}	0.10±0.1^{b}

Different letters in the same column indicate significant differences using Tukey HSD tests at the 5% level

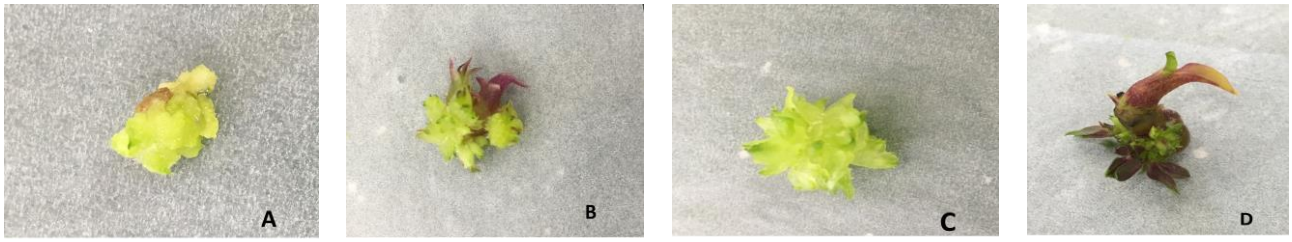

Figure 02. Effects of sucrose and trehalose on *Dendrobium k.* Jonathan's Glory 'Dark Joy' under different LEDs A: sucrose and white fluorescent lamps; B: sucrose and green LED; C: trehalose and white LED; D: trehalose and green LED.

IV. Discussion

The average number of PLBs and the amount of fresh weight are very important for successful and healthy PLB regeneration *in vitro*. LEDs are an efficient light source for plants (Manivannan et al., 2015; Lin et al., 2011) and play an important role in plant growth and development under *in vitro* conditions, depending on irradiation intensity (quantity of light), wavelength (quality) and light duration (photoperiod). Light is absorbed by chlorophyll (Topchiy et al., 2005). Carbon sources are another important factor in orchid PLB regeneration because it supplies energy to the plants especially when they are not ready to photosynthesize in the initial stage of growth (Al-Khateeb, 2008).

In this study, we used two types of carbohydrates; sucrose and trehalose, as carbon sources for two *Dendrobium kingianum* cultivars. Aggregation of trehalose plays an important role in protecting cell composition from damage by oxidation under stress conditions (Tokuhara and Mii, 2003). Moreover,

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trehalose itself can affect advancement by acting as a signal molecule in carbohydrate metabolism. The results of this study revealed that green LEDs with trehalose produced the highest average number of PLBs, but the maximum fresh weight was obtained with same light using sucrose in the medium for the *Dendrobium kingianum* 'Hallelujah' cultivar. A similar result has also been found for the growth of young tea plants, for which green LED was effective for the growth of potted and rooted cuttings (Homma et al., 2009) and also for strawberry plants, for which the growth and enlargement of the strawberry fruit plants were developed by green light irradiation (Kudo et al., 2009). Several studies have reported that orchid PLBs cultured under red LEDs showed the lowest differentiation rate while using blue LED resulted in the highest differentiation rate in cultures of *Oncidium* and *D. officinale in vitro* (Xu et al., 2009; Lin et al., 2011).

The results of the present study revealed that green LEDs and sucrose also increased the number of PLBs and amount of fresh weight of *Dendrobium k.* Jonathan's Glory 'Dark Joy', but trehalose decreased the number of PLBs and the amount of fresh weight under different LED for the same cultivar. Hew and Mah (1989) reported that carbohydrate hydrolysis by extracellular hydrolytic enzymes is possible, as demonstrated with the PLBs of *Dendrobium*. Carbohydrate is an important factor *in vitro* culture media and the appropriate amount is also important for the successful regeneration of PLBs. A deficient supply of carbohydrates for *in vitro* orchids can be detrimental to the cell growth rate. Sucrose is specifically needed in the plant embryo to increase cell division by encouraging cell expansion and reserve accumulation (Borisjuk et al., 2002). However, increasing sucrose over the threshold concentration could lead to excessive carbohydrate accumulation and hinder photosynthesis which eventually impairs the cell growth of rose plants (Capellades et al., 1991). The amount of carbohydrate is also responsible for good quality PLB formation. Sucrose (10 g/L) was found to be very inefficient in producing PLBs of *Dendrobium huoshanense* compared with 35 g/L sucrose system (Zha et al., 2007). Pulse treatments using trehalose and sucrose were considered to be useful for plantlet production in *Cymbidium* spp. (Shimasaki et al., 2003). Both carbohydrates and light are responsible for good quality PLB formation.

V. Conclusion

Micropropagation of plants has become a compelling technique for reproducing orchids that are otherwise difficult to propagate traditionally from seeds or vegetatively. This research showed that choosing an appropriate amount of carbohydrate with LEDs was effective for the organogenesis of PLBs of the *Dendrobium kingianum* cultivar. Based on this research and discussion, we conclude that green LEDs and trehalose promote the organogenesis of PLBs of *D. kingianum* 'Hallelujah' whereas Green LED and sucrose increased PLBs in *Dendrobium k.* Jonathan's Glory 'Dark Joy' within a short period. The results of this study also indicated that trehalose negatively affected PLB formation of *Dendrobium k.* Jonathan's Glory 'Dark Joy'. Further study is needed on the combination of different LEDs with different sources of carbohydrate for effective *in vitro* PLB organogenesis of *D. kingianum* 'Hallelujah' and *Dendrobium k.* Jonathan's Glory 'Dark Joy'.

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