- Short communication

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Effect of Light Emutting Diode (LED) Lamps and N-acetylglucosamine (NAG) on 0rganogenesis in Protocorm-like Bodies (PLBs) of a *Cymbidium* Hybrid Cultured *In Vitro*

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Authors investigated the effects of N-acetylglucosamine (NAG) under different LED lights (red, green, and blue) on organogenesis in protocorm-like bodies (PLBs) of a *Cymbium* hybrid cultured *in vitro*. Under lighting of green LED, addition of NAG at concentrations of 0.01, 0.1 and 1 mg/l had an potent effect of enhancing PLB numbers. Under red LED treatment, PLB cultures with NAG at concentrations of 0.01 and 0.1 mg/l also showed a higher number and higher rate of PLB formation. Shoot formation in PLB in fortified cultures was promoted under green and red LED light in presence of NAG. Blue LED light had a little effect on the shoot formation in PLB cultures. Fresh weight of cultures was the highest (250 mg) under green LED light in the medium containing 0.01 mg/l NAG. The results suggest that LED light source could be used as an energy efficient light source for control of organogenesis of *Cymbidium in vitro* and that the presence of NAG in red and green LED plays an important role in the proliferation of PLB and shoot formation.

Cymbidium species have been hybridized for over a century to produce plants with flowers of rich texture, color and size that have formed the basis of worldwide flower market. Thus, many attempts have been made to develop better methodologies for *Cymbidium* micropropagation. These hybrids are gaining in popularity in many countries, especially in Japan. Bartel et al. (2014) proposed a model in which the NAG oligosaccharides modify the architecture of the cell wall. To increase the efficiency of *in vitro* techniques, culturing conditions such as

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light, temperature, and medium composition must be optimized. The common light for *in vitro* cultures is provided by fluorescent lamp. Light emitting diode (LED) as a new light source have many advantages compared to fluorescent light, namely longer life, wavelength specificity and narrow bandwidth (Hoenecke et al. 1992, Brown *et al.* 1995). Red and/or blue light provided by LED lamps have been applied for promoting plant growth (Lee et al. 2007, Kim et al. 2004, Baque et al. 2010). Therefore, in this study the effects of NAG under different LED lights were investigated on the *in vitro* organogenesis in PLBs of a *Cymbidium* hybrid.

Protocorm-like bodies (PLBs) of *Cymbidium* Waltz 'Idol' were multiplied in modified MS (Shimasaki and Uemoto 1990) medium by transferring the new medium every two months. Approximately 5 mm long excised PLBs served as explants. Modified MS with quarter strength of ammonium nitrate (412.5 mg/l), half strength of potassium nitrate (950 mg/l), 20 g/l sucrose, and 2 g/l Phytagel (Sigma) was used as the culture medium. The pH of media was adjusted using 0.213 g/l 2-(n-morpholino) ethanesulfonic acid sodium salt (MES-Na) before autoclaving at 121°C for 15 min. N-acetylglucosamine (NAG) at concentrations of 0, 0.01, 0.1, 1, and 10 mg/l were added to the culture media before sterilization. Jars (250 ml UM culture bottle, Japan) with plastic caps containing 30 ml of the medium were used as culture vessels. Five explants were placed in each culture vessel and three culture vessels were used for each treatment. All cultures were maintained at 25°C under different light quality lamps (green LED, red LED, and blue LED) during 16 hrs photoperiod for 40 days. Experimental data were collected by counting the number of PLBs, shoots, and fresh weight. The data were statistically analyzed by calculating standard errors of the means (means ± SE, n = 15).

The growth and development of PLB cultures of *Cymbidium* Waltz 'Idol' were affected by different LED and NAG treatments *in vitro.* As shown in Table 1, the highest PLB formation rate (100%) was found in the culture media containing 1 mg/l NAG under green LED 0.01 and 0.1 mg/l NAG under red LED (Fig. 1A). A few PLBs formation were observed in the control, and in the presence of high concentration of NAG under green and red LED (Fig. 1B). Shoot formation (80%) was observed in the medium containing 0.1 mg/l of NAG under red light (Fig. 1C) and 1 mg/l under green light (Fig. 1D). The lowest shoot formation (10%) was found in the control of green LED (Fig. 1E). The maximum root formation (13.3%) was found in the medium containing 0.1 mg/l NAG under red LED, and the minimum root formation (6.6%) took place at a concentration of 1 mg/l NAG under green LED. In all other treatments there was no root formation indicating the effect of LED and NAG on organogenesis in PLB cultures. Fresh weight of PLBs was highest (250 mg) at 0.01 mg/l NAG under green LED.

Table 1. Effect of LED lamps and NAG on organogenesis in PLBs of C. Waltz *'Idol'* after 40 days of culture.

Treatments		PLB		Shoot		Root		
NAG (mg/l)	LED	Average number	Formation rate (%)	Average number	Formation rate (%)	Average number	Formation rate (%)	Fresh wt. (mg)
Control	Green	2.8±0.6	80.0	0.1±0.1	10.0	0	0	150
	Red	2.6±0.4	80.0	0.5±0.1	46.6	0	0	160
	Blue	2.2±0.4	80.0	0.2±0.1	13.3	0	0	110
0.01	Green	4.5±0.5	86.6	1.1±0.3	53.3	0	0	250
	Red	3.8±0.3	100	0.8±0.2	60.0	0	0	230
	Blue	3.4±0.5	86.6	1.2±0.4	53.3	0	0	200
0.1	Green	3.9±0.6	86.6	0.6±0.2	46.6	0	0	210
	Red	3.3±0.3	100	0.8±0.1	80.0	0.1±0.1	13.3	190
	Blue	2.9±0.4	93.3	0.2±0.1	20.0	0	0	110
1	Green	3.4±0.4	100	0.9±0.1	80.0	0.1±0.1	6.6	200
	Red	3.0±0.5	86.6	0.8±0.2	60.0	0	0	170
	Blue	3.0±0.3	93.3	0.1±0.1	13.3	0	0	180
10	Green	2.6±0.4	80.0	0.8±0.1	66.6	0	0	150
	Red	3.1±0.6	80.0	0.6±0.1	53.3	0	0	160
	Blue	2.6±0.4	93.3	0.2±0.1	26.6	0	0	110

Value represents means ± S.E. The cultures were examined after 40 days of culture. Each treatment consisted of three replicates, and each replicate consisted of five PLBs.

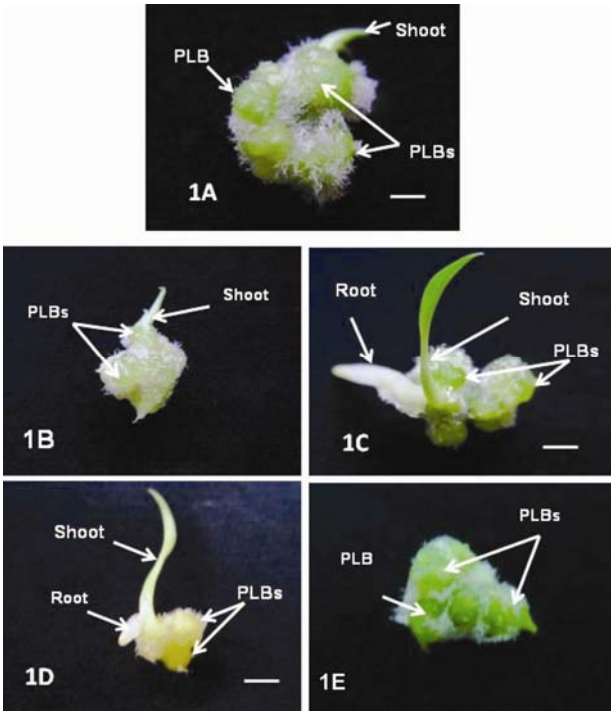

Fig. 1A-E. Development of protocorm-like bodies and shoot in a *Cymbidium* hybrid under different LED lights.

Light is one of the most important factors regulating plant development through photo receptors active under specific wavelengths of light (Lee et al. 2007). Orchid PLBs cultured under red LED showed lower productivity rate, while the application blue LED resulted in the enhanced productivity rate in cultures of *Oncidium* and *Dendrobium officinale in vitro* (Xu et al. 2009, Lin et al. 2011). The effects of green light on organogenesis and superoxide dismutase (SOD) activities in the formation of PLBs of *Cymbidium* cultured *in vitro* (Naruemol and Shimasaki 2012) have been recently reported. Another report describes the effect of different lights and two polysaccharides on the proliferation of PLBs of *Cymbidium* cultured *in vitro* (Nahar et al. 2011).

The results of our investigation revealed that both red and green LEDs in the presence of NAG in the media enhanced PLBs growth of a *Cymbidium* hybrid *in vitro* cultures.

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